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RESPONSES OF *APHIS FABAE* SCOP. TO WATER SHORTAGE IN HOST PLANTS IN THE FIELD

BY

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After two weeks of clear summer weather the water strain in naturally-rooted plants of *Euonymus europaeus* L. and *Beta vulgaris* L. was sufficient to reduce substantially the settling, larviposition and survival of *A. fabae* on mature leaves. These effects were more severe in *Euonymus* than *Beta* especially in conditions favouring rapid transpiration. A previous water strain on the other hand improved the host status of mature leaves during the recovery process, which was slower in *Euonymus*. The more drought-resistant properties of the woody host *Euonymus* help to make it resistant to colonisation by *A. fabae* in the summer, when herbaceous hosts such as *Beta* are preferred.

It was shown in the first of these papers (Kennedy, Lamb & Booth, 1958) that a considerable water strain in potted plants of Spindle (*Euonymus europaeus* L). and Broad Bean (*Vicia faba* L.) reduced feeding and larviposition by *Aphis fabae* Scop., while previous work on other aphids and plants had indicated that less acute or intermittent water strain in the host could have opposite effects, favouring aphid multiplication, in association with enrichment of the sap. The object of the field work here reported was to determine whether water strain sufficient to have unfavourable effects on the aphids, could occur in naturally rooted field plants as well. The spindle plant was of most interest here since we have never observed it to wilt under natural conditions. A further object was to compare the host status of spindle with that of a secondary herbaceous host, sugar beet (*Beta vulgaris* L.), when exposed to the same soil and atmospheric conditions, in view of the possible importance of their relative water status in the seasonal alternation of *A. fabae* between primary and secondary hosts.

Material and methods

The work was done entirely on plants in the 8 × 27 ft (2.6 × 8.3 m) raised garden bed described and figured by Kennedy & Booth (1951). A corrugated asbestos-cement partition was sunk to a depth of 3 ft across the bed so as to separate one third of it (9 × 8 ft) from the rest. At 1 a.m. on 30/6, and again at noon on 4/7 and 11/7, 1949, tap water to the equivalent of 1 in. (25 mm) of rain was sprayed on this section using a temporary screen to prevent spray reaching beyond the soil partition. In 1957, the soil in the same one-third bed section was kept near field capacity from the beginning of May onwards by watering it to the equivalent of 1 in. of rain whenever the reading of a Soil Moisture Tensiometer (porous pot 4 in. below the soil surface) approached 10 lb. per sq. in. The aphid and plant material, cages and experimental methods were as described previously (KENNEDY *et al.*, 1958).

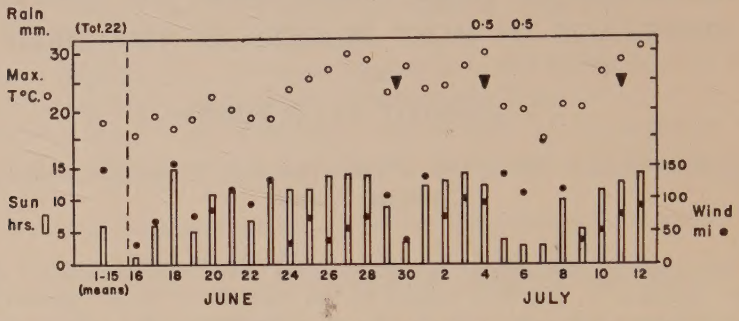

Fig. 1. Weather during 1949 experiments. Arrowheads above indicate occasions when one section of the bed was watered.

RESULTS

1. *Free colonisation of spindle*

As a direct counterpart of the experiment reported in the previous paper on free colonisation of potted spindles with reversal of the watering regime, observations were made concurrently on free colonisation by *A. fabae* of the spindles rooted in the raised bed before and after the watering of an end-section of it. Unlike the potted plants these spindles had not been pruned and their harder foliage never wilted visibly. By late June 1949, only a few sappy basal shoots were still growing and these were heavily infested by apterous adults and larvae (KENNEDY, IBBOTSON & BOOTH, 1950, p. 670). Otherwise the leaves were all mature and uninfested except for occasional alatae depositing small groups of larvae which remained under-sized and disappeared again in a matter of days, apparently through predation, before any became adult. Not more than 8 such grouplets were to be seen at any one time, and they were virtually absent from the plants at the more exposed, drier, north-east end of the bed. After this end had been watered on 30 June and 4 July, all the leaves on the bed were examined on 8 July when the soil surface was quite dry again and no new leaves had yet been produced by the arrested shoots. Table I shows that the watering had reversed the

TABLE I

Natural colonisation of watered and unwatered spindles

Bed section	Total shoots	Alatae per shoot	Groups of larvae per shoot	% groups with no alata
Watered	21	2.52	2.86	27
Unwatered	42	0.19	0.52	57

situation in terms of aphid incidence on mature leaves, repeating the results with the pot plants. The aphid incidence on the unwatered plants was about the same as before but there were now substantially more alatae and grouplets of their larvae on the watered plants, and fewer of these grouplets had been abandoned by their mothers. There were some 12% more leaves per shoot on the unwatered plants so the difference in terms of aphids per leaf was greater than Table I shows. It may be inferred that these naturally-rooted spindles were short of water at the time although their "hard" foliage gave no external sign of it.

2. *Cage comparison of watered and unwatered spindle and beet*

The above differences in incidence of natural colonies was simultaneously checked with single-leaf metal cages stocked with alatae from the greenhouse culture and attached to the mature spindle and beet leaves on the bed. Each cage was stocked twice, on 7 and 8 July, in the evening, and cleared of alatae the following evening. The larvae deposited by the first batch of alatae were left in place to be counted together with those deposited by the second batch. Table II shows that feeding, survival and larviposition were greater on the watered than the unwatered plants, both spindle and beet.

TABLE II

Mean percentages of alatae feeding and surviving, and mean number of larvae deposited per alata per day, on mature leaves of watered and unwatered plants. Based on 4 cages for watered spindle leaves and 5 each for the other types of leaf, with 10 alatae per cage. Cages re-stocked with fresh alatae at 18 hr. on 8 July.

Plant and bed section	8/7				9/7			
	feeding		surviving	feeding	feeding		surviving	larvae
	15.00	17.30	18.00	19.00	13.00	17.30	19.00	19.00
Spindle								
watered	11	42	82	61	19	11	86	1.36
unwatered	11	30	55	12	2	5	71	0.71
Beet								
watered	67	69	72	74	44	42	98	1.75
unwatered	5	25	59	59	11	48	75	0.46

The water strain appeared to affect spindle more than beet. Table II shows there was less settling and larviposition on the mature spindle leaves than on the mature beet leaves in the watered section of the bed, implying more water strain in the spindle leaves under these conditions even with ample water at the roots; and the same applied in the unwatered section apart from the afternoon of 8/7 when the beet leaves wilted badly. This contrasted with the experiments on watered pot plants in the greenhouse and most of the field experiments in the wetter year 1948, when the mature spindle leaves tended to be preferred to the mature beet (Kennedy & Booth, 1951, 1954 and below). Comparing the two days, there were 10 hours of sunshine and 113 miles run-of-the-wind on 8/7 against 5 hours (morning only) and 36 miles on 9/7 (Fig. 1). Accordingly, the mature leaves on the unwatered beet plants wilted noticeably on 8/7 but not on

9/7 when wilting was restricted to the senescent leaves. The aphids' settling responses varied in parallel, with fewer settled on the unwatered beet leaves relative to the watered, at 15.30 hr. on 8/7 than at 13.00 hr. on 9/7. But on the mature spindle leaves at 15.30 hr. on 8/7 the aphids settled equally and very little on the watered as well as the unwatered plants: confirming that the spindle leaves, unlike the beet, were under water strain even when amply watered in these conditions favouring rapid transpiration.

3. *Effects of ambient conditions on the difference between watered and unwatered spindle leaves*

That the host status of mature spindle leaves might be influenced by the ambient conditions affecting *inter alia* the rate of transpiration, as well as by the water supply from the roots, was tested directly at the same time. Ten mature spindle leaves on watered plants were each matched against a leaf on a neighbouring unwatered plant in two-leaf preference cages. The top covers of the cages were left open so as to expose to light and air the upper surface of the leaves under which the aphids were confined. Preference tests were made with fresh alatae on two occasions with the same pairs of leaves: first in dull cool weather (around 19.00 hr. on 7/7, or noon on 9/7), and then again in bright, warm, windy weather on the next or same day (around 17.00 hr. on 8/7, or 18.00 hr. on 9/7, respectively). In every cage the proportion of aphids settling on the watered leaf in preference to the unwatered one, was greater in the clear weather (60—96%) than in the dull weather (44—74%). Since there was no consistent difference between the two occasions in the total number of aphids settling, that is, no evidence of a difference in the strength of their feeding response, it seemed

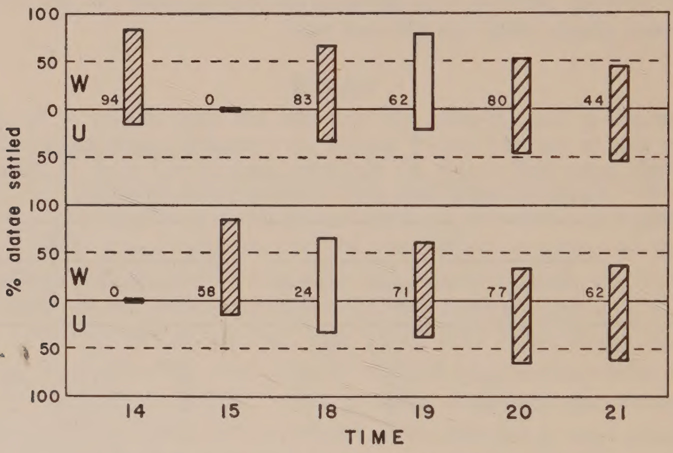

Fig. 2. Results of preference tests between watered (W) and unwatered (U) mature spindle leaves in two sets of 2-leaf cages, alternately exposed to the sun (open blocks) and shielded from it (closely-hatched blocks) until all were shaded from 19.30 hr. Each block shows the mean distribution of alatae in two cages on the same leaves throughout. Numbers against blocks show percentages of settled alatae among all those in the cage. Fresh alatae introduced at 13, 14.10, 17, 19.10 & 20.10 hr.

unlikely that the stronger preference for the watered leaves shown in the bright weather was due to a direct effect upon the aphids, such as they themselves being under greater " water strain". In order to minimize this possibility, five additional pairs of leaves were tested simultaneously on the same two occasions using alatae collected from the culture at the same time, but on the second occasion (sunny) a white metal shield was set 10—15 cm above each cage. This reduced the illumination to well below that of unshielded leaves in dull weather without impeding air circulation. In all five cages the shielding further reduced the proportion of aphids settling on the watered leaf, to below that recorded when the same leaves were unshielded in dull weather; again with no consistent difference in activity level.

In a second trial with the shields on 12/7, a clear, hot day (Fig. 1), the tests were started during the heat of the day. Of the four two-leaf cages used, two were shielded and two left exposed to the sun with subsequent alternation of this treatment. An hour was allowed after fixing the shields before taking the aphid distribution readings. The aphids introduced at 13.00 and 14.10 hr. died off quickly without settling in all the exposed cages; in the shade, they showed an overwhelming preference for the watered leaves (Fig. 2). At 18 and 19 hr. when it was cooler, fresh alatae showed a strong preference for the watered leaves when the cages were exposed to the sun, and the same aphids showed a weaker one when they were shaded: weaker also than had been shown in the shade earlier. Later still, when all the cages were unshielded but equally shaded by distant trees, the preference for the watered leaves disappeared altogether and fresh aphids now showed an opposite tendency to prefer the unwatered leaves. Concurrently, two single-leaf cages were set up on watered and two on unwatered leaves, one cage of each pair being shielded while the other was exposed, alternately. Fresh alatae were introduced into the cages at 14.30, 15.30 and 17.30 hr. and the settlers counted 30 min. later. These counts showed a sequence of changes in relative

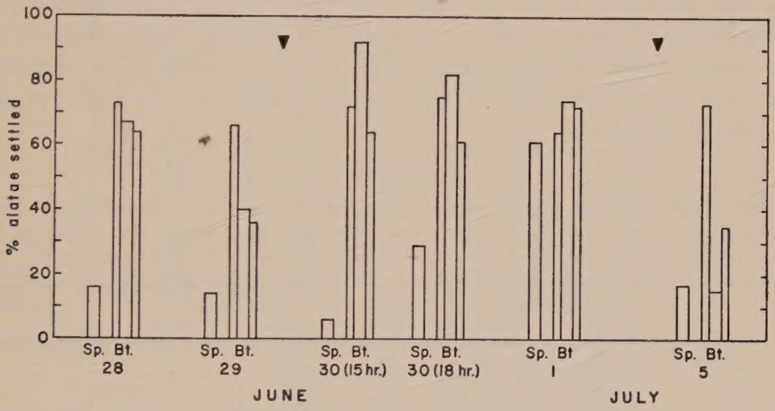

Fig. 3. Mean percentages of alatae (10 per cage) settled in the afternoon on 5 mature spindle leaves and 4 each young, mature and senescent beet leaves, shown from left to right in that order on each occasion. Arrowheads indicate watering as in Fig. 1.

settling on watered and unwatered leaves, like that in the two-leaf preference cages, including the final reversal in favour of the unwatered leaves after sundown.

4. Cage comparison of spindle and beet before and after watering

To complement the simultaneous comparison of spindle and beet, watered and unwatered, above (section 2), successive comparisons were made of spindle and beet before and after the plants were watered, testing the same individual leaves repeatedly. Single-leaf cages were stocked with fresh alatae in the late afternoon of 28/6, 30/6 and 4/7 and left for 48 hr. with periodic records of the number settled and a final count of alatae surviving and larvae deposited. In addition, the cages were stocked with fresh alatae for 2 hr. only in the early afternoon of 30/6, following the first watering at 1 a.m. that day.

On the days before and the first day of watering the alatae settled much less on the mature spindle leaves than on the beet leaves of all three ages (Fig. 3, 28—30/6). The numbers of alate surviving and of larvae deposited (Table III) confirmed the inferior host status of the spindle leaves on those days. But from the evening of the day of watering the host status of the spindles improved relative to the beets, the spindles apparently recovering from water shortage more slowly than the beets. By the last day, following a second watering on 4/7 and in duller weather (Fig. 1), settling had equalized between spindle and beet as far as the mature leaves were concerned (Fig. 3, 5/7). Larviposition and survival (Table III) were at this time as great on the mature spindle leaves as on the best (young) beet leaves.

TABLE III

Mean percentage of alatae surviving, and mean number of larvae deposited per alata, in successive tests on the same leaves before and after watering. Based on 5 cages on mature spindle leaves and 4 on each age of beet leaf, with 10 alatae per cage.

Leaf	28—29/6		30/6	30/6—1/7		4—5/7	
	surv.	larv.	surv.	surv.	larv.	surv.	larv.
Mature spindle	51	1.4	65	73	1.2	96	3.8
Young beet	63	3.9	92	88	1.6	95	3.9
Mature beet	60	3.4	98	95	2.2	81	2.9
Senescent beet	50	2.4	95	87	1.2	79	1.6

Among the beet leaves of the three ages feeding, larviposition and survival were greatest on the young leaves before watering, and least on the senescent leaves which wilted most. On the day of watering and that immediately following (30/6 & 1/7) the beets did not wilt, but settling, larviposition and survival all point to the mature leaves as the most favourable for the time being. At the last test on 4—5/7 the young leaves had spurted in growth and regained their superiority over the mature leaves as in normally watered plants. The status of the senescent leaves varied among the different measures used, these leaves having now aged past the point at which they are normally most favourable.

5. Effects of weather in 1948

The very inconsistent results previously reported (KENNEDY & BOOTH, 1951, pp. 43—5) from cage comparisons of spindle and beet on the raised bed in 1948, may now be re-considered. The weather during the summer of 1948 was unsettled except for the second half of July, as shown in Fig. 4 together with the detailed results of the six experiments covering June, July and August. The experiments were done as a rule in dry weather to avoid the risk of flooded cages. Winged aphids were rarely seen flying or starting new colonies in wet weather, and the relative acceptability of spindle and beet in wet weather did not, therefore, seem likely to have much bearing on the problem of host alternation in nature.

However, the first day of preference testing in Expt. H, 7/7, was exceptionally

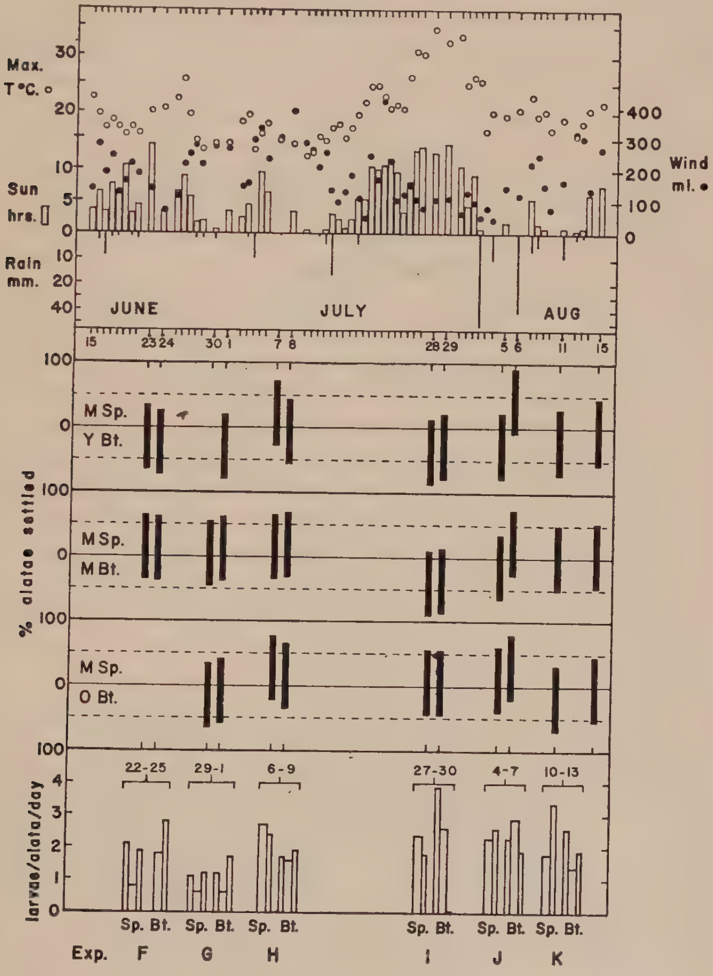

Fig. 4. Weather and results of experiments comparing spindle (Sp.) with beet (Bt.) through the summer of 1948 (after KENNEDY & BOOTH, 1951, Tables 9, 10, 17 & 18). Centre: results of preference tests, each block showing the mean distribution of alatae in 2—4 two-leaf cages in a total 6—12 tests; Y = young leaves, M = mature, O = senescent. Below: mean larviposition rates in 3—5 single-leaf cages on each leaf type; mature leaves shown as wide blocks, young leaves as narrow blocks to the left and senescent leaves as narrow blocks to the right of the wide blocks.

dull and humid (some aphids seen on the wing) and during the second day's tests in Expt. J, 6/8, it was actually raining (no aphids on the wing). On these particular days the alatae in the cages showed a strong bias toward spindle. They now settled on the mature spindle leaves in preference even to young and senescent beet leaves. And in both experiments, their preference shifted in the same sense with respect to the weather change from one day to the next, on the identical leaves. The preference for mature spindle over young and senescent beet leaves weakened or disappeared on the second day of Expt. H, which was brighter and drier than the first. The reverse preference shift with the reverse weather change in Expt. J was more striking, with a complete reversal from beet preferred to spindle preferred in the comparisons of mature spindle leaves with young and mature beet. The same reversal occurred in three additional cages (not shown in Fig. 4) included in this experiment to compare senescent spindle with young beet leaves. The preference shifts in this experiment were attributable to the changed atmospheric conditions only, since the soil surface was still damp on 5/8 after 50 mm of rain on 2/8 and 10 mm during the night of 4—5/8.

At the other extreme, at the height of the "heat-wave" in July when the beets were wilting badly every day, there appeared in Expt. I a strong bias away from the spindles in all comparisons with beet. Only the most wilted, senescent beet leaves were not strongly preferred to the spindle leaves; and in spite of the wilted state of these beet leaves the mature and to all appearances unwilted spindle leaves were barely preferred to them at this time, although distinctly preferred in the preceding and succeeding experiments H & J. The single-leaf cages (Fig. 4, bottom) also showed a drop in the host status of the spindle leaves relative to beet during Expt. I, now in terms of larviposition. In June (Expts. F & G) more larvae were deposited on young and senescent than on mature leaves of both spindle and beet. During the "heat-wave" the pattern changed in the same way on both plants: larviposition was now least on the senescent leaves, where in fact all the alatae were dead by the end of the first afternoon without having settled at all. Thus the senescent leaves seemed to be the most affected by water strain in both plants.

The fine weather ended on 2/8 and by the time of Expt. K, 10—13/8, the original pattern of least larviposition on mature leaves had been restored. But during the first few days after the return of wet weather there was a temporary improvement in the host status of the mature leaves, as in the 1949 experiment above. Thus in Exp. J. there was more larviposition on the mature beet leaves than on the now fully erect senescent ones, more even than on the young beet leaves. On the spindles larviposition was now insignificantly less on the mature leaves than on the senescent. An improvement in the mature leaves relative to other ages on both plants occurred also in Expt. H where again there had just occurred a change, if less marked, from drier to wetter conditions.

6. *Effects of previous water shortage in spindle*

More evidence of an improvement in the host status of mature leaves when water is supplied after a deficient period was sought in 1957, when it was possible to compare spindle leaves having such a history with others on the adjoining section of the raised bed in which the development of a soil moisture deficit had been largely prevented by watering (see Methods). May and June were generally fine and dry and by the end of June the tensiometer readings in the unwatered bed section were running between 40 and 50 lb. per sq. in. The plants there gave evidence of water deprivation in arrested growth, lower leaf water contents, paler leaves and lesser incipient colonisation by *A. fabae* migrants, compared with the plants in the watered section (these effects will be described in a separate paper). The tensiometer readings in the unwatered section had fallen to the same low level as in the watered section by 13/7, following 9 mm of rain on 11—12/7 which opened a period of dull wet weather lasting some two weeks. In the afternoon of 12/7, ten clip-on cages each containing one fresh apterous adult were attached to mature leaves on each section of the bed. The aphids were not observed to wander in the cages but did not live long (Table IV) as expected from previous work with "hard" mature leaves, and their progeny grew slowly into very small adults in both sets of cages. But the parents lived longer on the plants that had not been watered previously (P = 0.05). They also deposited more larvae before dying, but this difference was not significant statistically because, while they lived, the parents on the other plants, which had been watered previously, deposited their larvae more rapidly (not significant).

TABLE IV

Survival and larviposition on mature spindle leaves in wet weather after a long dry period. Means and standard deviations from 10 apterae caged singly

	Test plants: previously unwatered	Control plants: previously watered
Parent's survival, days	8.2 ± 5.1	4.0 ± 3.2
Total larvae per parent	13.9 ± 10.8	9.4 ± 7.1
Mean larvae/parent/day	1.6 ± 0.9	2.5 ± 1.5

DISCUSSION

Evidently the naturally-rooted plants used in these experiments did become subject to sufficient water strain in warm dry weather to reduce feeding, larviposition and survival by *A. fabae.* We suggested in the preceding paper that the opposite, favourable effects on aphids of water shortage in the host plant, are especially likely when plants are recovering after a water shortage and there occurs an outward "surge" of translocation from the leaves, enriching the phloem sap with breakdown products accumulated during the shortage (KENNEDY *et al.*, 1958). Some evidence of such a favourable effect was obtained with mature spindle leaves when rain terminated the dry period in 1957. And it is difficult to suggest any other explanation for the shift of feeding preference in favour of the unwatered mature leaves in the evening of July 12, 1949; and likewise, for the temporarily improved host status of mature spindle and beet leaves, relative to young and senescent ones, on the days immediately following the termination of water shortage in 1948 and 1949. The mature leaves had reverted to their normal inferior status on these watered plants by the time of the main daytime comparisons with unwatered plants (1949: sections 1, 2 & 3 above). Hence we attribute the superior host status of the watered over the unwatered mature leaves which was then found, not to the after-effects of previous water shortage (sap enrichment), but to the immediate difference in water status: the reduced turgor pressure or increased sap viscosity, or both, in the water-deficient leaves, presumably impeding the uptake of sap from them by the aphids.

The permanently erect appearance of mature spindle leaves in the field, and the generally deeper roots of trees and shrubs than of herbs, might suggest that spindles would be less subject to water strain than beets. Judging by the aphids' responses the opposite was in fact the case. The negative effects of soil water shortage on the aphids were greater in spindle than in beet; and even with ample soil water the spindle leaves seemed under greater water strain in atmospheric conditions favouring rapid transpiration. This accords with laboratory findings: (i) that detached spindle leaves surrender their water more freely than beet leaves; and (ii) that the external appearance of spindle leaves is no guide to their turgidity because the tissue shrinkage upon loss of water is largely internal, thanks to the more rigid structure of these leaves (KENNEDY & BOOTH, 1958). The more rigid structure of the leaves of woody plants is likely to mean, also, a greater loss of turgor for the same loss of water (*ibid.*), especially within the skeletal members (veins) where the aphids feed. Together perhaps with more viscous sap, this would mean much greater resistance to sap uptake through the aphid's narrow stylet canal, from mature spindle leaves than from mature beet leaves exposed to the same conditions of soil or atmospheric drought. These features will help to explain the scarcity of colonizing alatae on mature spindle leaves in fine summer weather — and that is when aphid dispersals tend to occur (JOHNSON, 1954). In wetter weather, when the soil moisture is restored and transpiration reduced, mature spindle leaves which have been under water strain may become for the time being highly favourable for colonisation by *A. fabae* (as on August 6, 1948); but then revert to an unfavourable state when rapid transpiration is resumed, or alternatively when the sap-enriching translocatory "surge" subsides.

Thus the drought-resistant properties of the mature leaves of the woody primary host, spindle, appear to make them especially aphid-resistant. This seems to be one reason for the preferential colonisation by *A. fabae* of herbaceous hosts such as sugar beet in the summer. A more important reason, since mature leaves are generally least favourable anyway, appears to be the relative absence of growing and senescing leaves on the woody host at that time (KENNEDY & BOOTH, 1951). But this feature, too, depends on water relations, a matter that will be dealt with elsewhere.

ZUSAMMENFASSUNG

Reaktionen von Aphis fabae *auf Wassermangel der Wirtspflanzen im Freiland*

1. Das Verhalten freilebender und gekäfigter virginoparer Geflügelter von *Aphis fabae* Scop. auf Blättern von *Evonymus europaeus* L. und *Beta vulgaris* L., die bei trockenem Sommerwetter zusammen in einem Gartenbeet wuchsen, wurde vor und nach künstlicher Bewässerung eines Teiles des Beetes beobachtet.

2. Besiedlung, Larvenproduktion und Überlebenstrate waren auf reifen Blättern unbewässer-

ter Plfanzen beider Arten niedriger. Doch waren diese Wirkungen der Wasserbelastung in der Wirtspflanze bei *Evonymus* stärker. Auch bei reichlicher Wasserversorgung der Wurzeln wurden die Aphiden auf *Evonymus* ungünstig beeinflusst, wenn die atmosphärischen Bedingungen rasche Transpiration begünstigten. Wenn sich die Pflanzen am Abend oder während der Tage unmittelbar nach der Bewässerung vom Wassermangel erholten, verbesserte sich zeitweilig die Wirtseignung der reifen Blätter im Vergleich mit dem Laub anderer Alterszustände der gleichen Pflanzen bzw. mit der reifer Blätter an Pflanzen, die vorher keinen Wassermangel erlitten hatten.

3. Die unterschiedlichen Ergebnisse, über die früher bei Feldkäfigversuchen zum Vergleich der Wirtseignung von *Evonymus* und *Beta* für *Aphis fabae* berichtet wurde, lassen sich in Übereinstimmung mit den vorliegenden Versuchen auf Witterungsänderungen zurückführen.
4. Die mehr auf Trockenresistenz ausgerichteten physiologischen Eigenschaften des holzigen Primärwirtes *Evonymus* scheinen sein Resistentwerden gegen Befall mit *Aphis fabae* bei Sommerwitterung zu unterstützen und damit die Abwanderung der Aphiden zu begünstigen.

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CORRELATION BETWEEN RAINFALL IN THE DRY SEASON AND THE OCCURRENCE OF THE WHITE RICE BORER (*SCIRPOPHAGA INNOTATA* Wlk.) IN JAVA

BY

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The amount of rain which falls sporadically in the relatively dry season at the time that the white rice borer is diapausing as a full grown larva in the rice stubble, has a very important influence on the size of population of the borers in the following wet season when rice is grown. If this dry season is wet or very wet (according to our standard, based on the figures of rainfall and borer damage over 26 years in five regions of Eastern Java), no damage can be expected in the following planting season. If the dry season is really dry or very dry, outbreaks of borers may occur (in the period under survey this occurred in nine out of fourteen years). We have tried to give an outline of a method of prediction of borer damage in the planting season, based on the rainfall dates of the foregoing dry season.

One of the most serious pests of rice (*Oryza sativa* L.) culture in Indonesia is the white rice borer (*Scirpophaga innotata* Wlk., Lep., Pyralidae). On several occasions outbreaks were followed by famine. Frequently, the outbreaks appear unexpectedly in places where for several years no damage has occurred. It would therefore be of great practical value, if some scheme of prediction of the occurrence of this borer could be given upon which a system of warning could be based.

The white rice borer is highly adapted to the climate of certain regions of Indonesia. The species occurs only in places where annually a distinct wet and dry season (Monsoon) occur, and where the average rainfall during October and November is not higher than 200 mm. At Java therefore, the occurrence of the white borer is limited to the coastal plains in the North and the level counties in the South East. No outbreaks are ever reported from the hill regions or the lands east of Surabaia, the so-called "Oosthoek".

The caterpillars are found to be monophagous, living on paddy only. Each year five to seven generations develop during the wet monsoon. At the end of this season, however, the grown-up larvae living in the maturing paddy bore downwards to the base of the stalk and enter into diapause. During the following dry season the caterpillars remain in the stubble for at least 4½ months. Activation of the larvae and pupation is caused by the first rains at the end of the dry season; four to six weeks later the moths appear. The (simultaneous) appearance of the first moth generation after the dry monsoon is called "stubbleflight".

It has already been known for several years that there are large fluctuations of borer damage in different years. The rice growers on Java speak of "borer years", years in which borer attacks are numerous. It is obvious that climatological circumstances must be the main cause of these differences, in particular the rainfall during the dry season[1]). In the wet season the reproduction of the borer is never seriously hampered by any factors, either biotic or abiotic. Several hymenopterous egg-parasites are known, but the level of parasitism is always low.

The mortality of the borers is highest during the time that the caterpillars are diapausing in the stubble, i.e. in the dry monsoon. Temporary moistening, caused by an occasional shower at that time, leads to the death of many borer larvae. In former years, it was recommended to control the pest by temporary inundation of the fields at the end of the dry season. It appears that interruptions of the dry season by rains, or a short dry season, may be unfavourable to the development of the borers.

Already DAMMERMAN (1915) tried to predict borer outbreaks based on the dates of rainfall during the years 1900—1913, and borer records during these years. Damage by borers might be expected if the dry season would be dry indeed while the following wet season begins late.

The nature of the diapause was investigated thoroughly by VAN DER GOOT (1925). Diapause begins independent of moisture, either in very humid weather conditions or under drier circumstances. Drying of the haulms had no influence. The old term for the diapause: "drought sleep", is wrong.

It was found by VAN DER GOOT that diapause could be induced by feeding the caterpillars with paddy in the pre-flowering stage or paddy which was developing ears. Feeding with younger stems did not induce diapause.

Mortality during the diapause was mainly caused by moisture. More or less regular rainfall in the first two months after the beginning of the diapause caused 100% mortality. Moistening at regular times in the third and fourth month of the diapause also caused the death of most caterpillars. It was clear from the investigations of VAN DER GOOT that after a relatively rainy dry monsoon no out-breaks of the white borers could be expected. On the other hand, prolonged drought had hardly any influence on the mortality of the diapausing larvae.

COMPARISON OF BORER DAMAGE AND RAINFALL, 1915—1940

Following up the references, mentioned above, an unfavourable effect of rainfall during the diapause period seems probable. However, exact dates showing the amount of rainfall and the months in which this influence is largest, were not given, and a scheme of prediction could therefore not be made. In this paper I have tried to compare the dates of borer damage and those of the rainfall, in order to try to find some correlation upon which a prediction of the occurrence of borer pests could be based. Some facts have been mentioned preliminarily elsewhere (VAN DER LAAN, 1958).

The basis for the comparison were the files and reports on both factors over 26 years (1915—1940) available at the Institute of Plant Diseases and Pests, at Bogor (Indonesia).

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[1]) The dry season of Java does not mean that there is no rainfall. Showers may fall at irregular intervals.

Borer damage. To begin with 1915, annual reviews of the occurrence of diseases and pests were published by the Institute of Plant Diseases and Pests at Bogor. These data, supplemented by those from the archives of this Institute, were evaluated and those which could be used for the present study, were retained. Initially eight districts in which borer damage occurred, both from western and eastern Java, were compared, but for the later years the western regions must be left out: In the most important region, Tandjong Brebes, the classic borer control by late sowing (VAN DER GOOT 1948; VAN DER LAAN 1951) has interrupted the natural development of borer populations there since 1935; in another place, Krawang, changes in the irrigation, started in 1931, influenced the appearance of white borers considerably.

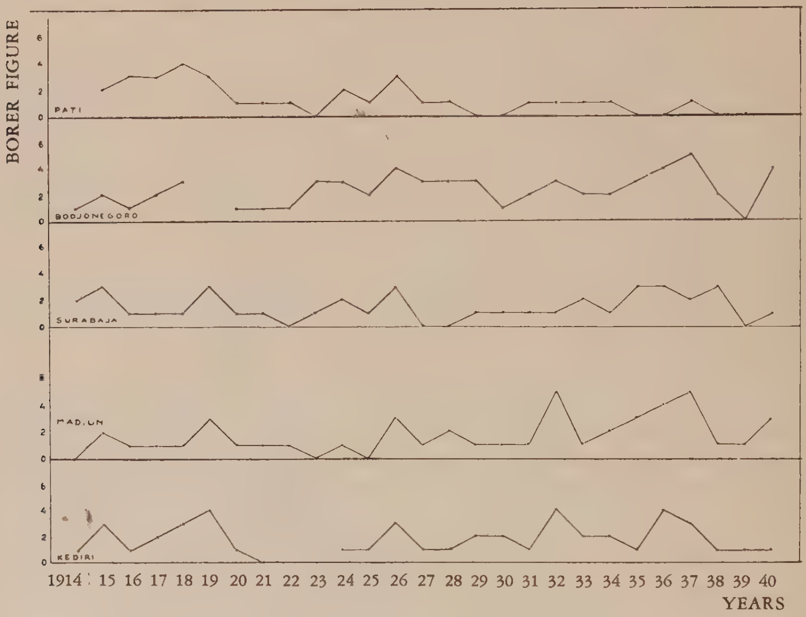

Fig. 1. Occurrence of the white riceborer (*Scirpophaga innotata*) in five regions in East Java, 1915—1940.

Five regions in East-Java were taken into consideration. Numerous data had to be left out because of inaccuracies in the reports. The greater the borer damage, the more significant the figures.

Rate of damage was classified in five groups:

5. Serious outbreak, many thousands of hectares afflicted, with complete failure, not only locally, but in several regions.
4. Borers occurring frequently, damage over 8 to 15.000 hectares per region.
3. Several outbreaks of borers, however more locally. Damage on 3 tot 8000 hectares per region.
2. Moderate attacks of borers, damage 1000 to 3000 hectares per region.
1. Borers were found to a small or very small extent, or the appearance was inconspicuous.
0. No occurrence of borers is mentioned in the reports.

Only these five groups were chosen. A more accurate estimate of borer damage in the separate regions cannot be made from the data available. The figures are shown in the graphs (fig. 1). The congruence of figures leads to the calculation of a "borer index" for every year. The indices are given in Table II (last column).

Rainfall. The figures for rainfall in the dry season during the years 1915 to 1940 for the same regions for which the figures of the borer damage were collected, were calculated from the meteorological data.

A formula was found (SCHMIDT & VAN DER VECHT 1952), to describe the rainfall character of the dry season.

From about November until April the rains fall at Middle and East Java. In the course of April the climate becomes drier though the rainfall never ceases completely. The dry season ends in the course of November.

This season was divided into three periods:

1. From the end of June backwards to the beginning of the dry season.
2. The months July and August (middle period).
3. From September 1st forwards until the end of the dry season.

A suitable criterion to indicate the beginning of the first period is found by the time which elapses from the end of June backwards, until 350 mm rain has been registered. The end of the third period occurs when 350 mm is found from September 1st forwards.

On this basis the average duration of the beginning (period 1) and of the end (period 3) of the dry season over 1915—'40 was determined (SCHMIDT & VAN DER VECHT, l.c.). These averages turned out to be 85 days for period 1, and 102 days for period 3. The yearly deviations in days of these averages were calculated. A negative figure for period 1 indicates a rainy period at the commencement of the dry season. A negative figure for the third period means an early end of the dry period, and thus also applies to a high rainfall level. For instance, if the figure + 25 is given to period 3 of a certain year, the dry season ended 25 days later than the average, the 350 mm were reached late and the period was dry.

TABLE I

Classification of separate years in groups according to the amount of rainfall in the dry season

Figure of group	Qualification	Criterion of beginning (period 1)	Criterion of July/Aug. (period 2)	Criterion of the end (period 3)
		in days	in %	in days
1	Very dry (early)	More than + 15	More than — 60	More than — 15
2	Dry (early)	+ 15 to + 5	— 60 to — 30	— 15 to — 5
3	Normal	+ 5 to — 5	— 30 to + 30	— 5 to + 5
4	Wet (late)	— 5 to — 15	+ 30 to + 60	+ 5 to + 15
5	Very wet (late)	More than — 15	More than + 60	More than + 15

The middle period was treated in a different way. The average rainfall was calculated and found to be 28 mm. From this the yearly deviations were calculated and expressed as percentages plus and minus as the case may be.

The periods were classified in groups as shown in Table I.

Following the classification in Table I, each year could be given a symbol of three figures: a year characterized by 4 - 2 - 3 had a wet start, a dry middle period and a normal end of the dry season.

In Table II the deviations and symbols of each year are given, together with the average figure of borer damage, whereas in Table III the years are grouped according to their borer index.

TABLE II

Yearly deviations from the average rainfall during the dry season in East Java, together with the mean figures of borer damage, 1915—1940

	First period (days)	Second period (%)	Third period (days)	Rainfall symbol	Average borer figure
1915	— 21	— 64	— 5	5-1-3	2,4
1916	+ 17	+ 132	— 40	1-5-5	1,4
1917	— 8	— 2	— 26	4-3-5	1,8
1918	+ 29	— 45	+ 12	1-2-2	2,4
1919	— 6	— 55	+ 2	4-2-3	3,2
1920	— 7	+ 204	— 38	2-5-5	1,0
1921	+ 21	— 27	0	1-3-3	0,8
1922	— 27	— 48	+ 6	5-2-2	0,7
1923	— 8	+ 204	+ 23	4-5-1	1,0
1924	+ 5	— 21	— 19	2-3-5	1,8
1925	+ 28	— 77	+ 24	1-1-1	1,0
1926	— 1	— 84	— 1	3-1-3	3,2
1927	— 13	— 30	+ 4	4-3-3	1,2
1928	+ 3	+ 11	— 4	3-3-3	1,4
1929	+ 27	— 98	+ 17	1-1-1	1,4
1930	+ 2	— 45	+ 6	3-2-2	1,0
1931	— 4	— 57	+ 10	3-2-2	1,2
1932	— 14	— 87	+ 4	4-1-3	2,8
1933	— 12	+ 32	— 15	4-4-5	1,6
1934	+ 2	— 20	— 8	3-3-4	1,6
1935	+ 7	— 93	+ 9	2-1-2	2,0
1936	— 8	— 37	0	4-2-3	3,0
1937	— 11	— 80	+ 5	4-1-3	3,2
1938	— 24	+ 132	— 4	5-5-3	1,4
1939	— 4	+ 354	+ 19	3-5-1	0,4
1940	+ 10	— 89	+ 16	2-1-1	2,2

+ = dry (early)   + = wet   + = dry (late)
— = wet (late)   — = dry   — = wet (early)

Discussion

Comparison of dates on borer damage and rainfall in Eastern Java (Table III) may lead to the following conclusions :

Favourable conditions for serious borer damage in the investigated regions of Eastern Java are found if:

1. The beginning of the dry season is wet (in one case normal);
2. The middle period is dry or very dry;
3. The end of the dry season has a normal amount of rain.

Conditions favourable for much damage by borers are as follows:

1. The beginning of the dry season is not relevant, may be wet or dry;
2. The middle period is dry or very dry;
3. The last period is normal, dry of very dry.

On the other hand, occurrence of borer damage is moderate, low or very low, under the following conditions:

TABLE III

Comparison of the figures of rainfall and the mean figures of borer damage in East Java, 1915—1940

Serious borer damage			Much borer damage		
	rain	*borers*		*rain*	*borers*
1919	4-2-3	3,2	1915	5-1-3	2,4
1926	3-1-3	3,2	1918	1-2-2	2,4
1932	4-1-3	2,8	1935	2-1-2	2,0
1936	4-2-3	3,0	1940	2-1-1	2,2
1937	4-1-3	3,2			

Borer damage locally			Borer damage sporadically			Borer damage very scarce		
	rain	*borers*		*rain*	*borers*		*rain*	*borers*
1917	4-3-5	1,8	1916	1-5-5	1,4	1921	1-3-3	0,8
1924	2-3-5	1,8	1920	2-5-5	1,0	1922	5-2-2	0,7
1933	4-4-5	1,6	1923	4-5-1	1,0	1933	3-5-1	0,4
1934	3-3-4	1,6	1925	1-1-1	1,0			
			1927	4-3-3	1,2			
			1928	3-3-3	1,4			
			1929	1-1-1	1,4			
			1930	3-2-2	1,0			
			1931	3-2-2	1,2			
			1938	5-5-3	1,4			

1. The amount of rain at the beginning period is not relevant;
2. The middle period is normal, wet or very wet;
3. The last period is wet or very wet.

Of the three periods taken into consideration, the first seems less important than the other two.

The above conclusions have restrictions. Borer damage does not always develop when rainfall figures are low. It is remarkable that in both very dry years (1—1—1), 1925 and 1929 the conditions were not favourable to the borers. Also the years 1930 and 1931 (both 3—2—2) caused no borer damage in the following planting season. No serious borer damage occurs in years following an extremely wet year (viz. 1920—1921).

Though it seems that the rainfall is a very important factor which influences the borer population, it is clear also that is is not the only factor. This follows also from the comparison of rainfall and borer figures of the years 1927 and 1932 (Table IV).

TABLE IV

Comparison in detail of two years with similar rainfall figures, but different borer dates in East Java

	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Borer date
1927	+ 29	+ 45	+ 44	— 15	— 11	— 28	— 31	— 25	1,2
1932	+ 25	+ 38	+ 54	— 36	— 26	— 17	— 5	— 18	2,8

Although the rainfall figures in both years have a close resemblance, there is great difference in borer attack.

The size of the borer population in the foregoing years will certainly be of some influence. Twice (1916—20 and 1934—38) a sequence of years occurs wherein the borer attack increases gradually. On the other hand, one rather wet year (1920, 1938) brings the series to an end, and often it needs several years before new outbreaks occur. Moreover, the influence of dry years is not always visible in the following season and is not apparent until the following year (viz. 1918, borer year in 1919; also 1925, borer year in 1926).

VAN DER GOOT (1925) pays some attention to the hymenopterous egg parasites (*Telenomus beneficiens* Zehnt., *Trichogramma australicum* Gir. and *Tetrastichus schoenobii* Ferrière). They have no diapause and survive the dry season in small number on other Pyralids. Parasitism on *Scirpophaga innotata* never reaches high levels for that reason.

Prediction of borer outbreaks

Is it possible to give a prediction from these figures, when an outbreak of the white borer could be expected ? This would be of great practical advantage. In that case a prediction might be given some months before the borers appear in the rice plantations, based on the rain figures of the foregoing dry season. In the first place the opinion of the older authors is confirmed, that dry seasons, which are relatively wet or very wet (in the sense used in this paper), *never* were followed by borer outbreaks in the next planting season in the 26 years, which are surveyed here.

If the middle period is dry or very dry, it is probable that an outbreak will occur. In the years investigated, 14 such years occurred, and 9 of them were followed by outbreaks. The exceptions have different causes. One of these is that extreme dry years are not followed by borer pests.

As for the third period, if the last months of the dry season are normal or dry, borer outbreaks may occur. If this period is wet or very wet, borers appear occasionally, no severe infestations being recorded.

A scheme of prediction should be based in the first place on the rainfall in the middle period, the months July and August. The average rainfall in the region under consideration should be determined.

No serious outbreaks of borers can be expected if the rainfall in July and

August exceeds this average. Even if the rainfall is less than 30% of the average figure, only local damage can be expected.

There is a real chance that borer damage will occur if the rainfall in this period is 30% or more below the average. For instance, if the average is 28 mm, an amount of moisture of 20 mm or less may be cause outbreaks.

Secondly, the last period of the dry season is important. The average number of days which elapses from September 1st until 350 mm rain is found, should be determined. If in the year for which the prediction is made, the amount of 350 mm is reached within 80% of the average time, there is no chance of serious borer damage. If the 350 mm-level is reached within an average of 100 days, there will be no damage if this level is already reached in 80 days or earlier.

On the other hand, if the 350 mm-level is be reached after more than 80% of the average time, borer outbreaks may occur in the following rice planting season.

Thirdly the beginning period of the dry season does not give so much help for the purpose of prediction. If the 350 mm-level (from June 30th backwards) is reached soon, and this pre-season is wet, there is more chance that borers occur then in the case when this period is relatively dry.

Following these rules, and within the limits of the available data from 26 years only, the certainty of prediction that no — or only local damage of small proportions — will occur is 100%. On the other hand, the certainty that real outbreaks can be predicted, is no more than 64%.

ZUSAMMENFASSUNG

Beziehung zwischen Regenfall in der Trockenzeit und dem Auftreten des Weißen Reisbohrers (Scirpophaga innotata *Wlk.) in Java*

Der Weisse Reisbohrer (*Scirpophaga innotata* Wlk.) kommt nur in Gebieten vor, wo jährlich eine ausgeprägte Regen- und Trockenzeit (Monsun) auftritt. Am Ende der Regenzeit gehen die vollerwachsenen Raupen, die im Stengel der reifenden Reispflanze leben, in Diapause. Während der folgenden relativ trockenen Jahreszeit, wenn nur gelegentlich Regenschauer fallen, ruhen die Raupen in der Stoppel. Bald nachdem die ersten heftigen Regenfälle der neuen Regenzeit einsetzen, verpuppen sich die Larven und alle Falter schlüpfen gemeinsam zum „Stoppelflug".

Dieser Zünsler ist in gewissen Gebieten Javas einer der Hauptschädlinge des Reises, obwohl sein Auftreten sehr unregelmäßig ist. Jahrelang war man allgemein der Auffassung, daß relativ reichlicher Regenfall während der Trockenzeit die Reisbohrerpopulation erheblich vermindere und daß in diesem Falle keine schweren Schäden durch Reisbohrerbefall erwartet werden können.

Während eines Zeitraums von 26 Jahren (1915—1940) wurden in fünf Gebieten Ost-Javas sowolhl der Niederschlag wie der Bohrerschaden untersucht und Berechnungen der jährlichen Niederschlagsmenge in der Trockenzeit und des Bohrerschadens verglichen. Dies erhärtete die alte Auffassung, daß nach einer relativ feuchten oder sehr feuchten Trockenzeit kein Bohrerbefall von Bedeutung während des Beobachtungszeitraumes auftritt. Dagegen folgten in neun von 14 Jahren mit einer trockenen oder sehr trockenen Trockenzeit schwere oder ziemlich schwere Ausbrüche der Weißen Reisstengelbohrer während der folgenden feuchten Jahreszeiten. Obwohl auch andere Faktoren von einiger Bedeutung sein dürften, wird geschlossen, daß die Niederschlagsmenge während der Trockenzeit einen sehr entscheidenden Einfluß auf die Möglichkeit des Auftretens einer Reisbohrer Kalamität im darauffolgenden Jahre hat. Diesen Daten entsprechend wird auf der Grundlage der Niederschlagszahlen in der vorangegangenen Trockenzeit ein Prognose-Schema entwickelt, wann Bohrerschaden zu erwarten ist.

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A STUDY OF THE FLIGHTS HABITS OF THE EUROPEAN CHAFER

BY

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By using a large cage in the field and a system of marking, the flight activities of 36 females and 49 males of the European chafer were followed during June and July, 1954.

It was found that the beetles made one to 11 flights during their life span with males averaging five flights and females 4.5. More males made eight to 11 flights than did females, and males made more flights on consecutive evenings. The average longevity of the males was found to be six days while the females lived on the average for 6.5 days.

The main flight period of the European chafer lasts about one month though some beetles can be observed flying in the evenings for about two months after the main flight has ended. The populations observed in the field are composed of successive groups of beetles which emerge, fly for several days then die. These groups overlap each other so that high numbers of beetles are present in the trees each evening during the main flight period. Variations in the physical characteristics of the soil habitat could be responsible for the differences in the time of maturity of the larvae and the subsequent "first-time" emergence of the adults.

In the United States, the biology of the European chafer, *Amphimallon majalis* (Razoumowsky) has been described by SCHWARDT & WHITCOMB (1943) and GYRISCO, WHITCOMB, BURRAGE, LOGOTHETIS & SCHWARDT (1954). They found that the biology of this scarabid differed in the United States in the length of its life cycle and feeding habits than that described for it by REGNIER (1935, 1936 & 1939) and AULLO-COSTILLO (1929) in Europe. REGNIER reared the beetles from eggs to adult reporting that in Normandy, France they had a two-year life cycle. In the United States GYRISCO *et al.* (1954) have found that the European chafer, as a rule, has a one-year life cycle although a small percentage may pass through a second year. AULLO-COSTILLO (1929) reports that in Spain the adults are considered as destructive pests of conifers. In the United States, the adults do not feed except possibly in trace amounts.

One of the most interesting but little known phases of the biology of the European chafer, *Amphimallon majalis* (Razoumowsky), is the flight activity of the adult. Such studies are fundamental to any applied investigations on distribution and rapidity of spread for the purpose of setting up quarantines in infested areas. Papers by SCHWARDT & WHITCOMB (1943) and GYRISCO *et al.* (1954) describe its crepuscular mating flights. BURRAGE (1951, 1952) and GYRISCO *et al.* (1954) investigated the sex ratio of adults participating in such flights and in preliminary experiments found that the adults were capable of making at least four flights. EVANS & GYRISCO (1958) describe the influence of light intensity on nocturnal emergence and flight; a manuscript on other work completed by them on orientation and distance of flight is now in preparation. This paper is concerned with total number of flights of the two sexes, frequency of adult emergence and adult longevity.

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MATERIAL AND METHODS

In general, the procedure was to mark a number of adults confined in a large cage in the field in such a manner that particular colors applied to specific parts of the body indicated the dates on which the beetles had emerged and flew. By using this marking system from the day the first beetles appeared until the last, it was possible to learn the frequency of flight and the approximate length of life of each individual beetle, and by identifying each specimen, differences in activity which might be related to sex or age were noted. Since some beetles may have lived in the soil after their last flight and died there, it was not possible to determine the exact life span of any individual beetle.

Early in May 1954 a wire screen cage measuring nine feet long, six feet wide and nine feet high was buried in the soil to a depth of four feet. The soil was leveled and packed inside to correspond to the height of the soil outside of the cage and a small shrub was planted within. In this way the confined beetles could enter and leave the soil without escaping and could swarm and mate on the screens of the cage and on the shrub.

On June 19, 1954, a mixed collection of 150 pupae and callow adults, representing a sample of the chafer population in the area, was dug from the soil in a nearby field and was placed in the soil within the cage. Of this number, 85 beetles made one of more flights from June 19 to July 19. Each evening during this period, beetles which emerged from the soil were captured and marked with a spot of "airplane dope" type paint or fluorescent paint on a particular part of the body by using a grass stem as a stylus. These paints were not toxic to the beetles and had been tested previously for retention of colour and for their ability to remain adhered to the cuticle. As the beetles were captured, records were kept of the sex and of previous markings of each one so that it was possible to follow the frequency of flight of each of the 85 beetles.

TABLE I

The number of crepuscular flights made by male and female beetles of the European chafer in a field cage. McKenzie Gravel Field, Clifton Springs, New York. June and July, 1954.

No. of flights	No. of females	Per cent of total	No. of males	Per cent of total
1	3	8.3	5	10.2
2	3	8.3	8	16.3
3	4	11.1	5	10.2
4	8	22.2	5	10.2
5	8	22.2	5	10.2
6	5	13.8	10	20.4
7	4	11.1	5	10.2
8	0	0	0	0
9	1	2.7	5	10.2
10	0	0	0	0
11	0	0	1	2.0
Total	36		49	

Discussion

As may be seen in Tables I and II, the number of flights made by the beetles ranged from one to 11 with a median number of 5.0 for the males and 4.5 for the females. Six males, or about 12 per cent of the sample of male beetles, made eight to 11 flights, while only about three per cent or one female made eight or more flights. Only 14 per cent of the females made seven or more flights, while nearly 25 per cent of the males made seven or more. These data are in agreement with field observations where large numbers of sexed beetles were marked and released in sex ratio studies (Gyrisco *et al.*, 1954). Recoveries on subsequent days always yielded a greater proportion of males, indicating that males fly more frequently than females.

TABLE II

A comparison of the flight activities and longevity of males and females of the European chafer in a field cage. McKenzie Gravel Field, Clifton Springs, New York. June and July, 1954.

Description of activities	Male	Female
Median number of flights	5.0	4.5
Mean number of flights	4.7 ± 0.37	4.4 ± 0.32
Percentage of adult life spent emerged at night	78.8	65.4
Mean number of two or more consecutive days on which flight took place	3.4	2.78
Median longevity in days	6.0	6.5
Mean longevity in days	6.0 ± 0.49	6.8 ± 0.57

The data in Table II also show that the males spend more time emerged and make more consecutive flights than the females. Since the beetles do not feed, the flight activity is directed toward mating, and presumably, females spend more

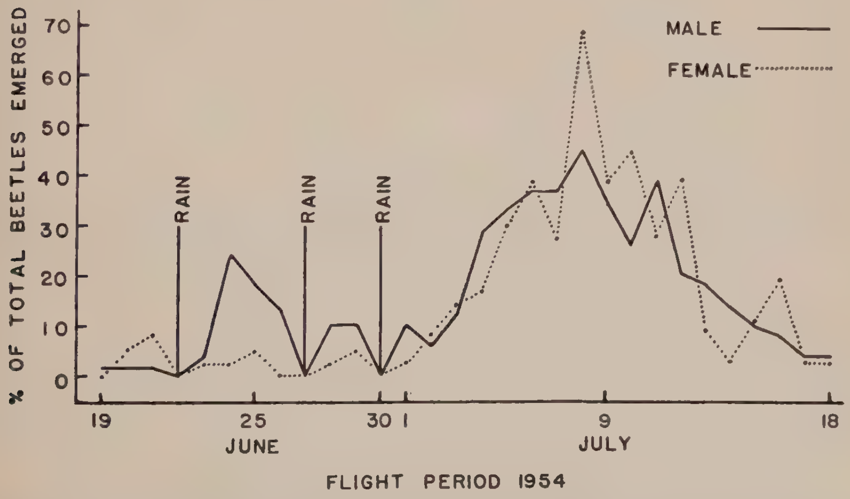

Figure 1. The percentage of males and females of the European chafer which emerged nightly in a field cage. McKenzie Gravel Field, Clifton Springs, New York. June—July, 1954.

time in the soil in order to construct egg cells and to deposit eggs. Individual males have been observed mating nightly over a period of several days. It should be noted, however, that individual females, while mating less frequently, also were found to mate more than once.

Figure 1 illustrates the frequency of flight of all of the beetles in the field cage during the flight period of 1954, and it is apparent that, although the males flew more frequently than the females on an individual basis, the total flight activities of both sexes tended to be synchronized over the entire flight period. More males than females emerged early in the season, but a peak of emergence of all beetles occurred in the middle of July with females outnumbering the males on several occasions. There was no emergence on June 22, 27 and 30 when strong winds and heavy rains prohibited flight activity. Light showers or even heavy storms of short duration just prior to flight time, however, did not interfere with normal emergence and flight.

TABLE III

The longevity of male and female beetles of the European chafer in a field cage. McKenzie Gravel Field, Clifton Springs, New York. June and July, 1954.

Longevity in Days	No. of females	Per cent of total	No. of males	Per cent of total
1	3	8.3	5	10.2
2	1	2.7	6	12.2
3	4	11.1	4	8.2
4	2	5.6	1	2.0
5	4	11.1	6	12.2
6	4	11.1	6	12.2
7	1	2.7	3	6.1
8	2	5.6	7	14.3
9	7	19.4	5	10.2
10	1	2.7	2	4.1
11	5	13.8	1	2.0
12	2	5.6	1	2.0
13	0	0	1	2.0
14	0	0	0	0
15	0	0	1	2.0
Total	36		49	

Figure 1 shows the activity of the total number of beetles in the cage over a period of 30 days, but as Tables II and III show, the average longevity of the males was 6.0 days, while for the females it was 6.5 days. (The data on the length of life of the adults were based on the duration of flight of each beetle and are exclusive of the callow resting period succeeding the pupal stage.) Hence, over a 30-day period successive groups of beetles emerged, flew several times and died. "First-time" emergence of individual beetles occurred from June 19 to July 11, but during that period the greatest number of individuals emerged for the first time from July 3 to July 9. Thus, during the entire flight period essentially different populations were present even though they overlapped each other.

From these observations it is believed that natural populations of the adults of the European chafer exhibit this same pattern of successive populations overlapping each other during the flight period. From the middle of June to early July different groups emerge so that large numbers of adults are present in the trees every night, even though the active life span of each beetle averages about six or seven days. Mass flights may occur for a month or longer, while very limited numbers of beetles have been observed during their crepuscular flights from the end of July to Mid-October (Gyrisco *et al.*, 1954).

The time of emergence of any beetle is dependent on the type of location in which the larval life was spent. The length of the larval period is believed to be influenced by the various physical characteristics associated with the soil habitat, such as soil type, the amount and kind of soil cover, the drainage in the area, the elevation, the protection from or exposure to the prevailing winds and finally the direction in which the area faces when on the slope of a hill. Under these varying conditions the larvae mature and emerge as adults at different times. The variations in time of emergence and the subsequent overlapping of successive groups of beetles result in a main flight period of the European chafer lasting about a month with a peak of emergence in early July and with a few beetles emerging on through early October.

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ZUSAMMENFASSUNG

Studien über die Fluggewohnheiten von Amphimallon majalis *(Razoumowsky)*

Im Juni und Juli 1954 wurde die Flugtätigkeit von 36 weiblichen und 49 männlichen Tieren von *Amphimallon majalis* (Razoumowsky) unter Zuhilfenahme eines grossen Käfigs im freien Felde und eines geeigneten Bezeichnungssystems der Tiere verfolgt.

Es zeigte sich, dass die Käfer während ihrer Lebensdauer ein bis elf Flüge unternehmen und zwar betrug der Durchschnitt für männliche Tiere fünf, für weibliche 4,5 Flüge. Mehr Männchen als Weibchen flogen 8 bis 11 mal und die Männchen flogen häufiger an aufeinanderfolgenden Abenden. Für männliche Käfer war die durchschnittliche Lebensdauer 6 Tage, während die weiblichen einen Durchschnitt von 6,5 Tagen erreichten.

Die Hauptflugperiode von *Amphimallon majalis* erstreckt sich über etwa einen Monat, obwohl man auch 2 Monate lang nach der Hauptflugzeit des abends noch einige Käfer fliegen sehen kann. Die Gesamtzahl der im Versuchsfeld beobachteten Insekten setzt sich aus auf einander folgenden Gruppen von Käfern zusammen, die nach einander ausschlüpfen, mehrere Tage fliegen und dann sterben. Diese Gruppen überschneiden sich, so dass während der Hauptflugzeit abends eine grosse Anzahl von Käfern in den Bäumen zu finden ist. Es ist sehr wohl möglich, dass Unterschiede in der physikalischen Beschaffenheit des Bodens für die Länge der Reifezeit der Larven verantwortlich sind und somit auch für den Zeitpunkt des Erstfluges der entwickelten Käfer.

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ERHOEHTER VIRUSBEFALL IN DEN RANDREIHEN VON KARTOFFELBESTAENDEN ALS FOLGE DES FLUGVERHALTENS DER VEKTOREN

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Versuchsparzellen von Ackersegen-Kartoffeln in Nord- und Mitteldeutschland wurden durch wöchentliche Insektizidbehandlungen während der gesamten Vegetationsperiode 1956 frei von Blattlauskolonien gehalten. Prüfung des Nachbaus auf Virusverseuchung 1957 ergab, daß die Bestände 1956 dennoch Virusinfektionen erhalten hatten, daß der Grad der Verseuchung mit der örtlichen Flugintensität der Vektoren (*Myzus persicae* Sulz.) in enger Beziehung stand und daß dabei die Randreihen bedeutend mehr Infektionen aufwiesen als die Mitte der Bestände. Diese Befunde werden mit dem Flug- und Wirtswahlverhalten der geflügelten Vektoren interpretiert.

Die intensive Beschäftigung verschiedener Autoren mit dem Verhalten phytopathologisch bedeutsamer Blattläuse bei der Wahl ihrer Wirtspflanzen (MOERICKE, KENNEDY und Mitarbeiter, H. J. MÜLLER & UNGER u.a.) hat im Laufe der letzten zehn Jahre auch zu einer experimentell begründeten Vorstellung über das Zustandekommen des erhöhten Randbefalls geführt, der an Beständen verschiedenster Kulturpflanzen eine bekannte und häufige Erscheinung darstellt (H. J. MÜLLER 1953b, 1957). Die Verdichtung des Aphidenbesatzes auf den Bestandsrändern entsteht bei der Primärbesiedlung der Felder durch die geflügelten Migranten in erster Linie dadurch, daß die Läuse während der sogenannten Befallsflugphase unter ökoklimatisch geeigneten Flugbedingungen auf der Suche nach neuen Wirtspflanzen ganz dicht über dem Boden dahinfliegen und — durch den Farbkontrast zwischen Pflanze und Erdboden, vielleicht auch durch die Silhouetten der Pflanzen, zur Landung veranlaßt — mehr oder weniger zwangsläufig auf die Phalanx der Randreihen geraten. Je dichter der Bestand geschlossen ist, umso weniger dringen sie auch bei den anschliessenden Probierflügen in das Innere des Bestandes vor. Nur solange die Bestände jung sind oder die Pflanzen aus anderen Ursachen sehr locker stehen, sickert der Initialbefall auch in die Feldmitte ein, so daß die Befallsdifferenz zwischen Rand und Mitte umso geringer zu sein pflegt, je früher die erste Befallsflugwelle das Feld trifft und vice versa.

In ähnlicher Weise wie der Aphidenbefall zeigt nun auch die Virusverseuchung der Kartoffelfelder in den Randpartien oft einen deutlichen Anstieg, wie zuletzt u.a. durch v. BERNUTH 1949 gezeigt wurde. Da seit langem nachgewiesen ist (KÖHLER 1954), daß bei der Verbreitung der meisten Kartoffelvirosen die Aphiden, insbesondere die Grüne Pfirsichlaus, *Myzus persicae* Sulz., als Vektoren die ausschlaggebende Rolle spielen und da sich neuerdings die Erkenntnis durch-setzt, daß dabei den geflügelten Stadien, insbesondere den allochthonen Zuwanderern die Hauptbedeutung zukommt, liegt es nahe, die erhöhte Randverseuchung der Kartoffelfelder durch Abbauvirosen mit dem Wirtswahlverhalten der geflügelten *Myzus persicae* in Zusammenhang zu bringen, obwohl erhöhter Besatz der Randreihen mit *Myzus persicae* infolge der größeren Unruhe und versteckten Siedlungsweise der Grünen Pfirsichläuse selten so deutlich in Erscheinung tritt wie beim *Aphis fabae*-Befall anderer Kulturpflanzen. Es kommt aber bei der Übertragung insbesondere der nonpersistenten Viren neben der Tätigkeit der nicht flugfähigen Mitglieder der größeren Kolonien vor allem auf die mehr oder weniger kurzfristigen Besuche und auf die besonders infektiösen Probesaugstiche der geflügelten Vektoren beim Befallsflug an, der oft gar nicht oder erst nach häufigem Pflanzenwechsel zur Ansiedlung bzw. zur Bildung von Initialkolonien führt (KENNEDY 1950, H. J. MÜLLER & UNGER 1952, 1955, H. J. MÜLLER 1953b).

Daß das Verhalten von *Myzus persicae* beim Befallsflug dem von *Aphis fabae* Scop., bei der die oben dargestellten Zusammenhänge im wesentlichen erarbeitet wurden, weitgehend ähnelt, hatten H. J. MÜLLER und H. J. MÜLLER & UNGER für die Quedlinburger Abbaulage bereits früher nachgewiesen. Für die Mecklenburger Hochzuchtlage bestätigt eine im August 1953 durchgeführte mehrtägige Versuchsreihe des erstgenannten Autors die Feststellung, daß *Myzus persicae* beim Befallsflug die bodennächsten Luftschichten fast noch stärker bevorzugt als *Aphis fabae*. Wie Tabelle I zeigt, fingen die Gelbschalen umso mehr *Myzus persicae*, je näher sie am Boden aufgestellt waren. So wurden in 20 cm Höhe im Mittel noch nicht halb so viele geflügelte Pfirsichläuse erbeutet wie unmittelbar an der Bodenoberfläche und in 50 cm Höhe betrugen die Fangwerte nur knapp $^1/_3$, in 120 cm

TABELLE I

Anzahl der Pfirsichläuse (Myzus persicae), *die sich in Groß-Lüsewitz an 12 Tagen (1953) in Höhen von 0 bis 175 cm über dem Erdboden fingen.*

Datum	0 cm	8 cm	20 cm	50 cm	120 cm	175 cm
9.8.	21	16	6	2	2	0
10.8.	95	60	35	32	20	9
11.8.	11	7	12	3	1	0
13.8.	18	17	11	11	5	1
14.8.	94	45	22	23	14	3
15.8.	46	62	27	17	11	1
16.8.	17	12	3	5	0	2
18.8.	35	32	13	10	3	2
19.8.	13	7	6	3	5	1
20.8.	35	39	30	19	2	3
21.8.	5	1	2	0	1	0
22./23.8.	9	9	6	1	1	1
X	33,3	25,6	14,4	10,5	5,4	1,9
P %*		16,3	1,9	0,5	0,4	0,4

* Die Sicherungswerte wurden nach der Differenzmethode bei paarweiser Zuordnung der Einzelwerte errechnet, wobei jeweils die höhere Schale mit der O-Schale verglichen wurde.

Höhe nur noch $^1/_6$ der am Boden ermittelten. Auch an den Randreihen eines mehr oder weniger erwachsenen Kartoffelbestandes wird also der Befallsflug von *Myzus persicae* gleichsam aufgefangen und — sofern es sich um virustragende Blattläuse handelt — zu gehäuften Infektionen führen, während im Zentrum des Feldes der Infektionsdruck wesentlich geringer sein dürfte.

METHODE

Im Rahmen einer vergleichenden Analyse der Populationsdichte und Befallsflugintensität von *Aphis fabae* und *Myzus persicae* in Kartoffel-Hochzucht- und Abbaugebieten (H. J. MÜLLER & UNGER, NEITZEL & RAEUBER, MOERICKE & SEEMANN 1959), wurde zum Nachweis der dargelegten Vorstellungen versucht, in Groß-Lüsewitz und Quedlinburg vergleichbare, primär virusfreie Bestände einer virusanfälligen Kartoffelsorte so anzulegen, daß allein durch geflügelte Aphiden Infektionen erfolgen und im Nachbau geprüft und lokalisiert werden konnten.

Benutzt wurde die Sorte Ackersegen. Für jeden Ort wurden 550 etwa gleich große Knollen dieser Sorte ausgelesen, von jeder Knolle ein Auge ausgeschnitten und im Augenstecklingstest in Groß-Lüsewitz auf Virusbefall vorgetestet. Auf diese Weise wurden sämtliche viruskranken Knollen entfernt, so daß je Versuchsort ein volkommen virusfreier Bestand von 500 Stauden erstellt werden konnte. Außerdem wurden je Versuchsort 25 Knollen in große Töpfe gepflanzt, um die im Laufe der Entwicklung doch noch eventuell auftretenden sekundär kranken Stauden aus dem Bestand entfernen und die dadurch entstandenen Lücken mit gleichaltrigen Pflanzen ausfüllen zu können. Die 500 Knollen wurden in Quedlinburg am 7.5.1956 im Verband von 60 × 40 cm und in Groß-Lüsewitz am 25.4.1956 im Verband von 62,5 × 36,5 cm mit 20 Reihen zu je 25 Knollen ausgepflanzt. Beide Bestände hatten also eine nicht ganz quadratische Form von 10 × 12 bzw. 9 × 13 m. Die einzelnen Pflanzstellen wurden numeriert und in einen Pflanzplan eingetragen.

Etwa eine Woche nach dem Auflaufen beginnend (Quedlinburg ab 2.6., Groß-Lüsewitz ab 28.5.1956) wurden die Bestände in Abständen von 5 Tagen mit einer 0,3% Wofatox-Lösung (Phosphorsäure-Ester) bis zur volkommenen Benetzung der Stauden gespritzt. Diese Behandlung wurde bis zum Absterben der Pflanzen fortgesetzt. Damit sollte bei weitgehender Ausschaltung der ungeflügelten Vektoren festgestellt werden, wie stark und welcher Art der Einfluß der Befallsflugintensität auf die Neuerkrankung eines gesunden Ausgangsbestandes ist; denn die massierten Insektizidbehandlungen mussten ja einerseits jedes längere Verweilen der Aphiden oder gar Initialkolonienbildung durch Abtötung unmöglich machen, während sie andererseits kurzfristige Besuche und Probesaugstiche der befallsgestimmten Aphiden voraussichtlich nicht stören konnten, da der Erfolg selbst der am raschesten wirkenden Insektizide bekanntlich nicht schnell genug eintritt um sie zu verhindern.

Die Ernte erfolgte in Quedlinburg am 2.11.1956 und in Groß-Lüsewitz am 1.11.1956; in beiden Fällen nach einem Nachtfrost, der auch die teilweise noch grünen Randstauden vernichtet hatte. Von jeder Staude wurden 5 Knollen entnommen und in eine Tüte gebracht, die mit der entsprechenden Staudennummer

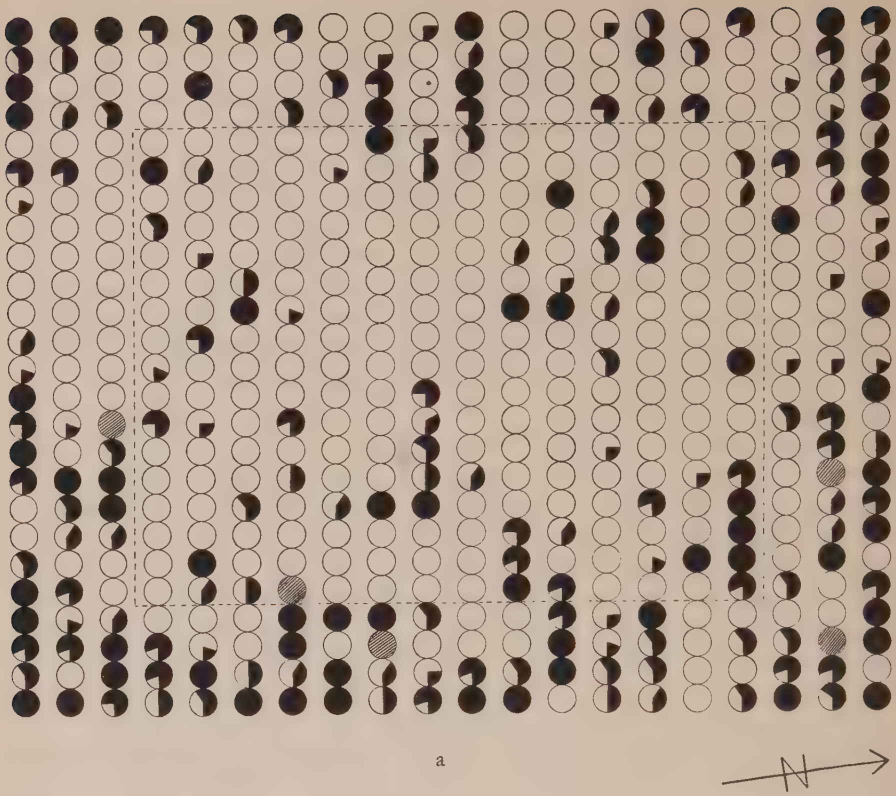


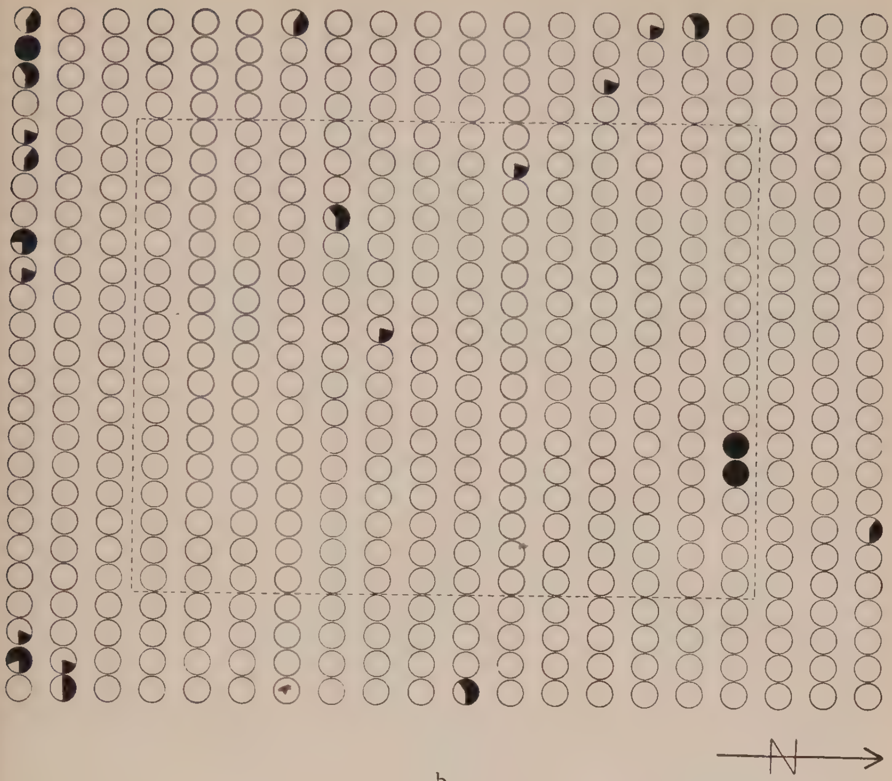
Abb. 1. Reproduktion des Pflanzplanes der Kartoffelversuchsparzellen des Jahres 1956, a. in Quedlinburg, b. (nächste Seite) in Groß-Lüsewitz. Im Nachbau (1957) erkrankte Pflanzen mehr oder weniger schwarz, gesunde weiß gekennzeichnet (weitere Erläuterungen im Text).

versehen war. Das Material beider Orte wurde in Groß-Lüsewitz eingelagert und 1957 staudenweise nachgebaut. In zwei Bonitierungen, am 27.6 und 18.7.1957, wurde der Virusbesatz dieses Nachbaus (Blattroll, Strichel) festgestellt. Auf diese Weise war es möglich, den Standort jeder einzelnen Staude aus dem Vorjahr (Infektionsjahr) zu reproduzieren und den Gesamtvirusbesatz an den beiden Orten sowie die Verteilung der Infektionen im Bestand zu ermitteln.

Der allgemeine Verlauf der Befallsflugintensität von *Myzus persicae* wurde während der gesamten Vegetationsperiode 1956 an beiden Orten mit Hilfe von Gelbschalenfallen verfolgt, die in der unmittelbaren Umgebung der Kartoffelparzellen auf Bracheparzellen aufgestellt und täglich geleert wurden.

Ergebnisse

Das Ergebnis des Nachbaues ist in der Abbildung 1 aufgrund der Pflanzpläne der beiden Ackersegenparzellen dargestellt, wie sie in Quedlinburg und Groß-Lüsewitz im Infektionsjahr 1956 zum Anbau kamen. Jeder Kreis stellt eine Staude



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dar, wobei die schwarz ausgefüllten Kreise oder Ausschnitte die im Jahre 1956 erfolgten Neuinfektionen (Blattroll, Y) wiedergeben.

Waren alle 5 nachgebauten Knollen der Staude erkrankt, so ist der Kreis schwarz ausgefüllt, waren nur 1 bis 4 Knollen erkrankt, so ist der Kreis nur teilweise schwarz ausgefüllt. In wenigen Fällen konnten auch nur 3 oder 4 Knollen nachgebaut werden, da die restlichen 1 oder 2 Knollen faul waren. In diesem Falle wurde bei der Darstellung sinngemäß verfahren. Die Knollen von den 5 schraffiert dargestellten Stauden konnten infolge Fäulnis nicht nachgebaut werden; sie wurden durch andere ersetzt.

Zunächst ist zu erkennen, daß an beiden Versuchsorten mehr oder weniger zahlreiche Virusinfektionen erfolgt sind. Da sich auf beiden Parzellen während der gesamten Vegetationszeit bei eingehenden Kontrollen niemals Blattlauskolonien auffinden ließen, die Entstehung sessiler Populationen also durch die wiederholten Wofatox-Behandlungen wirksam verhindert worden ist, müssen die Infektionen in erster Linie auf die Tätigkeit allochthoner Geflügelter zurückgeführt werden. Daß sie auf der Zuwanderung Ungeflügelter beruhen, ist höchst unwahrscheinlich, zumal sich eine enge Korrelation zwischen der Befallsflugintensität von *Myzus persicae*, dem Hauptvektor, und dem Grad der Virusverseuchung ergibt (s.u.). Der vorliegende Versuch ist also u.a. ein weiterer eindrucksvoller Beweis dafür, daß sich die Virusverseuchung auch durch regelmäßige und gegenüber sessilen Aphiden voll wirksame Insektizidbehandlungen nicht ausschalten läßt, weil sie offensichtlich die Infektionen durch die hauptsächlich wirksamen kurzfristigen Besuche der geflügelten Vektoren nicht verhindern können.

Infolgedessen kommt auch im vorliegenden Vergleichsversuch erwartungsgemäß der übliche starke Unterschied in der Virusverseuchung der Kartoffeln zwischen Gesundheits- und Abbaulage zum Ausdruck. Der Gesamtvirusbesatz des Nachbaues der Quedlinburger Parzelle beträgt 27,9%, der der Groß-Lüsewitzer nur 2,3%, d.h. im mitteldeutschen Trockengebiet am Rande der Magdeburger Börde war die Virusverseuchung ca. 12 mal stärker als im Mecklenburgischen Küstengebiet.

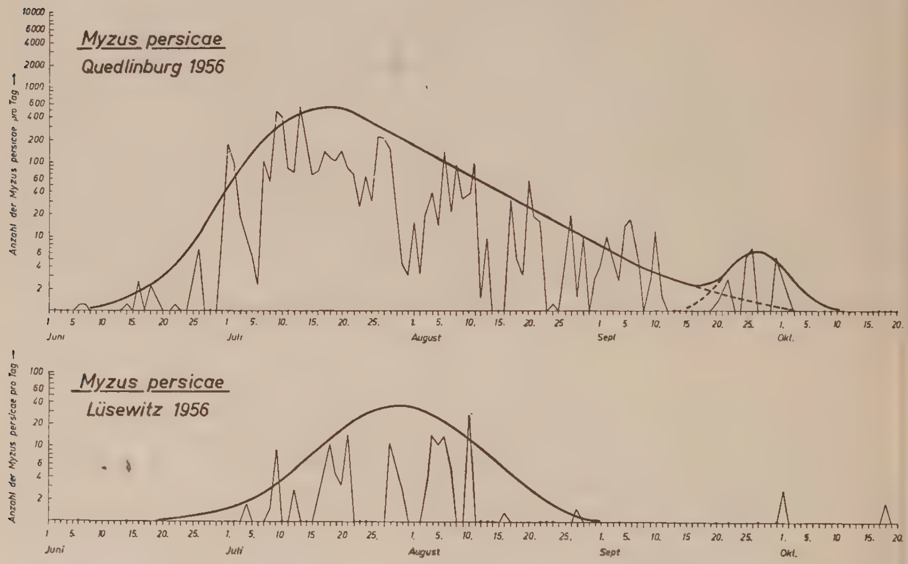

Abb. 2. Verlauf der Intensität und Dauer des Befallsfluges von *Myzus persicae* im Jahre 1956 in Quedlinburg (oben) und in Groß-Lüsewitz (unten) nach täglichen Gelbschalenfängen (Mittelwerte von je zwei auf Brache stehenden Schalen). (Nach H. J. MÜLLER et al. 1959, im Druck).

Legt man der Beurteilung alle Pflanzen zugrunde, bei denen im Nachbau überhaupt Virus nachgewiesen werden konnte, so ergeben sich im ganzen in beiden Fällen zwar höhere Verseuchungsprozentsätze (43,6% in Quedlinburg und 4,4% in Groß-Lüsewitz), doch bleibt das Verhältnis annähernd das gleiche, d.h. der Virusbefall beträgt in der Gesundheitslage etwa nur 10% des in der Abbaulage festgestellten.

In deutlicher Parallelität dazu steht die Befallsflugintensität von *Myzus persicae* während der Vegetationsperiode der Kartoffeln, die an beiden Orten Ende Mai aufliefen und in den ersten Novembertagen geerntet wurden. Während sie in Quedlinburg von Anfang Juni bis Anfang

Oktober dem Befallsflug täglich praktisch pausenlos ausgesetzt waren, erhielten sie in Groß-Lüsewitz nur von Ende Juni bis Anfang September einen spärlichen, häufig von mehrtägigen Pausen unterbrochenen Zuflug (siehe Abb. 2). Intensiverer Flug setzte zwar an beiden Orten ziemlich gleichzeitig Anfang Juli ein, nachdem die Bestände schon weitgehend herangewachsen und mehr oder weniger geschlossen waren, doch erreichten die täglichen Fänge in Quedlinburg (um den 10. Juli) Werte von fast 500 *Myzus persicae* pro Schale, in Groß-Lüsewitz aber höchstens ca 15. Insgesamt wurden zwischen dem 1. Mai und dem 1. Oktober in Quedlinburg 4718, in Groß-Lüsewitz im gleichen Zeitraum aber nur 162 *Myzus persicae* pro Schale erbeutet, d.h. also nur 3,4%. Bis zu welchem Grade diese starke Differenz auf Unterschieden der Populationsdichte einerseits und der witterungsbedingten Agilität der flugfähigen Vektoren andererseits beruht, wird in der oben zitierten Untersuchung (H. J. MÜLLER et al.) näher analysiert. Hier ist nur die Feststellung von Interesse, daß Befallsflugintensität und Virusverseuchung in Quedlinburg um mehr als eine Zehnerpotenz über den entsprechenden Größen in Groß-Lüsewitz lagen und — da ungeflügelte Vektoren ausgeschaltet waren — offensichtlich in einer engen Korrelation zueinander stehen.

Schon bei oberflächlicher Betrachtung der Pflanzpläne (Abb. 1) ist — besonders bei der stärker verseuchten Quedlinburger Parzelle — festzustellen, daß die Mehrzahl der Infektion in den ersten zwei bis drei Randreihen erfolgten, während in der Feldmitte der Infektionsdruck deutlich geringer gewesen sein muß. Teilt man die Parzellen in zwei etwa gleiche Teile: in Rand (außerhalb der Markierung in Abb. 1) und Zentrum (innerhalb der Markierung) auf, so erhält man für die Randbezirke der Quedlinburger Parzelle einen Virusbesatz (Blattroll, Y) von 39,4% und für das Zentrum einen von 15,3%. Die entsprechenden Werte für die Groß-Lüsewitzer Parzelle betragen 3,1 und 1,3%. Die Virusverseuchung war also bei diesen beiden sehr kleinen Schlägen mit ihren relativ großen Rändern in der äußeren Hälfte etwa zweieinhalb mal so stark als in der inneren. Es ist auffallend, daß trotz der großen Unterschiede im Gesamtbefall

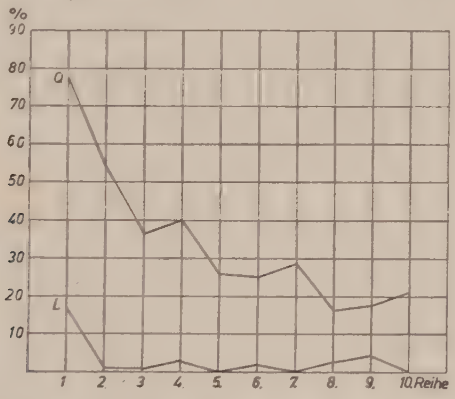

Abb. 3. Die Abnahme der Virusverseuchung von außen nach innen, gemessen am prozentualen Anteil (im Nachbau) infizierter Stauden in den einzelnen „konzentrischen" Reihen der Versuchsparzellen in Quedlinburg (Q = oben) und in Groß-Lüsewitz (L = unten).

das Verhältnis zwischen Rand und Mitte an beiden Orten nahezu gleich ist. Auch das ist wohl nur mit dem Wirken des gleichen Prinzips, d.h. mit dem Verhalten der Vektoren während des Befallsfluges zu erklären, wobei die Dichte der Flugpopulation keine Rolle spielt.

Am deutlichsten wird die Abnahme des Virusbefalls von außen nach innen, wenn für jede der aufeinanderfolgenden (sozusagen „konzentrischen") Pflanzenreihen der Prozentsatz abbaukranker Pflanzen getrennt ermittelt wird, wie es in Abb. 3 aufgrund der Ergebnisse des Nachbaues geschehen ist. Dabei wurde jede Pflanze als krank angesprochen, wenn wenigstens eine der vier oder fünf nachgebauten Knollen einen Symptomträger lieferte. Dabei zeigt sich eine rasche Abnahme des Befalls bereits in den zwei bis drei äußeren Reihen. In Groß-Lüsewitz ist der Erkrankungsprozentsatz bereits in der zweiten Reihe nicht mehr höher als in allen weiteren nach innen folgenden.

In größeren Schlägen wird der Unterschied vermutlich noch stärker sein als in den kleinen Versuchsparzellen, da sich bei letzteren nach innen ein sekundärer, rein geometrisch bedingter Konzentrationseffekt kompensierend auswirkt, indem sich die von allen Seiten zufliegend zu denkenden Vektoren nach der Mitte zusammenscharen und, soweit sie hierher vordringen, wieder zunehmend mehr Infektionen bedingen.

In erster Linie ist aber für das Verseuchungsgefälle die Standweite der Pflanzen bzw. der Bestandsschluß zur Zeit der ersten starken Befallsflugwellen entscheidend; denn wie bereits oben angedeutet und von H. J. Müller 1957 genauer ausgeführt, dringen die Vektoren umso tiefer in die Bestände ein, je breiter die freie, unbedeckte Bodenfläche zwischen den Pflanzen noch entwickelt ist. Im vorliegenden Falle waren mit Einsetzen konzentrierter Befallsflüge von *Myzus persicae* Anfang Juli die Bestände noch nicht völlig geschlossen, infolgedessen ist das Verseuchungsgefälle noch nicht so steil wie es häufig zu beobachten ist (v. Bernuth 1948).

TABELLE II

Die Abnahme der Verseuchungsquotienten Rand: Mitte bei Kartoffelbeständen mit zunehmend späterem Pflanztermin, berechnet nach den Virusprozentsätzen in der graphischen Darstellung (Abb. 2) v. Bernuth's *1948.*

	Blattroll			a-Mosaik		
	Rand	Mitte	Quotient	Rand	Mitte	Quotient
8.5.	16,0%	4,0%	4,0	12,5%	1,0%	12,5
15.5.	20,0%	10,0%	2,0	15,5%	2,5%	6,2
22.5.	31,0%	18,0%	1,7	24,0%	5,5%	4,4
29.5.	40,5%	41,0%	0,9	19,5%	8,0%	2,4

Einen besonders eindringlichen Beweis für die Abhängigkeit des Verseuchungsgefälles vom Bestandsschluß liefert der Pflanzzeitenversuch v. Bernuths (1948) mit vier Pflanzterminen im Mai, wobei der Nachbau von stichprobenweise entnommenen Rand- und Mittepflanzen auf Blattroll- und a-Infektionen bonitiert wurde. Wie die nach den Diagrammwerten v. Bernuths aufgestellte Tabelle II zeigt, gleicht sich der Verseuchungsunterschied zwischen Mitte und Rand mit zunehmend späterem Pflanztermin immer mehr aus. Während am 8. Mai gepflanzte Kartoffeln in den Randreihen 4 mal mehr Blattroll- und 12,5 mal mehr a-Mosaikinfektionen aufweisen als in der Bestandsmitte, zeigen am 29. Mai gepflanzte hinsichtlich Blatrollinfektionen keinen Unterschied mehr zwischen Rand und Mitte und beim a-Mosaik erhielt der Rand dann nur noch 2,4 mal mehr Infektionen als die Mitte.

Diese zunächst etwas befremdlichen Ergebnisse werden sofort verständlich, wenn man bedenkt, daß vom Pflanzen bis zum Auflaufen der Kartoffeln in der Regel 3—4 Wochen vergehen und bis zum vollen Bestandsschluß noch weitere 4—5 Wochen. Während der nach der Darstellung v. BERNUTHs am 8. Mai gepflanzte Bestand Ende Juni schon gut entwickelt war, lief der am 29. Mai gepflanzte erst auf. Ende Juni ist aber der Zeitpunkt, an dem in der Regel die Intensität des Befallsfluges stark ansteigt und in manchen Jahren, wie 1957, in Bernburg schon das Maximum des Befallsfluges erreicht wird. Der am 8. Mai gepflanzte Bestand muß dann also die typische Randwirkung zeigen, weil er schon weitgehend geschlossen ist, während in dem zuletzt gepflanzten, gerade erst auflaufenden, die gesamte geflügelte Population einsickern kann, der Infektionsdruck sich also gleichmäßig über den ganzen Bestand auswirkt, wobei die sonst typische Randwirkung nicht in Erscheinung tritt. Bei den mittleren Pflanzdaten muß sich dementsprechend eine gewisse Abstufung im Erkrankungsgrad zwischen Rand und Mitte einstellen, wie es die Darstellung v. BERNUTHs sehr deutlich demonstriert.

Wahrscheinlich ist auch die in den Ergebnissen von LEMKE (1950) und APPEL (1952) angeführte stärkere Erkrankung der Randreihen und die Abnahme der Anzahl erkrankter Pflanzen nach der Mitte des Feldes zu in ähnlicher Weise zustande gekommen.

Für die Praxis der Pflanzguterzeugung ist aus diesen Feststellungen der Schluß zu ziehen, die ersten 3—4 Randreihen sowie mindestens die ersten 4 Stauden in der Reihe von der Anerkennung auszuschließen, besonders bei weniger virusresistenten Sorten.

Desgleichen dürften sich kleine, besonders lange und schmale Feldstücke, bei denen der Anteil der ersten 3—4 Randreihen an der Gesamtfläche relativ groß ist, ungünstig auf die Pflanzgutqualität auswirken. Im vorliegenden Fall (eines allerdings sehr kleinen Feldstücks) stieg der Virusbefall bei der Parzelle in Quedlinburg von 15% (ohne Randbezirke) auf 28% (mit Randbezirken), also um rund 100%. Selbst wenn, was oft der Fall ist, die kleinen schmalen Flächen zunächst von Getreide umrahmt sein sollten, wird dieser Schutz doch Ende Juli/Anfang August mit dem Schnitt des Getreides wirkungslos und das zu einer Zeit, in der in den meisten Jahren die Hauptflugzeit von *Myzus persicae* noch nicht abgeschlossen ist. Wenn auch die frühen Sorten zu diesem Zeitpunkt mehr oder weniger abgereift sind, so bilden doch die noch weitgehend grünen, mittelspäten und späten Sorten den anfliegenden Aphiden ausgezeichnete Infektionsmöglichkeiten, zumal die Randstauden oft bis in den Spätherbst grün bleiben. Obgleich zu dieser Zeit schon eine gewisse Altersresistenz bei den Pflanzen vorhanden sein mag, gelangt sicher bei starkem Infektionsdruck noch ein großer Teil des Virus in die Knollen.

Die bekannte Forderung der Praxis nach frühest möglichem Pflanztermin und schneller Jugendentwicklung der Pflanzkartoffelbestände läßt sich also aus diesen Zusammenhängen zwanglos erklären. Aus den gleichen Gründen versagen unter unseren Verhältnissen Spätpflanzungen in den meisten Jahren, weil sie in der Regel bald nach dem Auflaufen, d.h. solange sie noch nicht geschlossen sind, in das Maximum der *Myzus persicae*-Befallsflüge geraten, die dann, statt vorwiegend nur die Randpflanzen, alle Pflanzen des Schlages — noch dazu in einem sehr infektionsempfindlichen Stadium — treffen und mit Infektionen beladen können.

Für die Hilfe bei der Überwachung und Betreuung des Quedlinburger Bestandes danken wir Herrn Dipl. Biol. K. BEHRENDT herzlich.

SUMMARY

Increased virus infection on the edges of potato plots effected by the flying behaviour of the vectors

In the course of comparative investigations of the significance of the infestation flight of *Aphis fabae* Scop. on the distribution of virus diseases, the virus infection which is frequently different on the edges and in the centre of virus diseased potato fields, has been analysed in the potato degeneration area (i.e. where seed potatoes cannot be grown year after year due to the high incidence of virus) of Middle Germany (Quedlinburg) and in the seed growing area of Northern Germany (Groß-Lüsewitz, Mecklenburg). In 1956 a nearly square plot of 500 healthy Ackersegen plants was kept free from apterae (aphids) by a weekly treatment with insecticides (Wofatox spray) and in 1957 the following generation (from 5 by 5 tubers taken separatedly from each bush) was tested for virus infection. The infection was found to be by 12 times more frequent in the degeneration area than in the seed production district. This can only have been due to the visits of alate vectors, especially *Myzus persicae* Sulz., the infestation flight of which was 29 times greater in Middle than in Northern Germany. In both places the virus infestation in the test plots was observed to decrease quickly from edge to centre. At Quedlinburg the third row showed barely one half of the infestation of the edge and the 5th row only one third of the infestation of the outer rows. At Groß-Lüsewitz it dropped to a tenth in the second row. This phenomenon is due to the nature of the infestation flight of the vectors, which glide extremely near to the ground. The flight is stopped at the bordering rows because of the density of the crop, effecting a far higher infestation rate here than in the centre, where it penetrates only very sparsely. This was evident from an experiment on planting times, described by v. BERNUTH 1948: The later the potatoes grow up, the smaller is the virus infestation quotient edge to centre, for when the plants are all small the whole crop is equally exposed to the infestation flight of the vectors, which begins by the end of June.

From this experience the demand of agricultural practice for the earliest possible planting and quickest possible covering of the ground by crop is be easily unterstood.

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THE INFLUENCE OF THE MANURIAL TREATMENT OF ORCHARDS ON THE POPULATION DENSITY OF *METATETRANYCHUS ULMI* (KOCH) (ACARI, TETRANYCHIDAE)

BY

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The influence of the nitrogen-content of apple leaves on the development of the fruit tree red spider mite (*Metatetranychus ulmi* (Koch)) was studied under laboratory and semi-field conditions.

The experiments in the laboratory showed that a higher egg production per female in 24 hours, a lower mortality rate of the eggs, larvae and nymphs, and a shorter period of development of the male as well as the female mites was obtained on the leaves with a high nitrogen-content. However, these differences were statistically significant only in the case of egg production and mortality rate. From the experiments under semi-field conditions it became evident that the egg-female ratio on leaves with high nitrogen content originating from manured rootstocks was also significantly higher than this ratio on leaves with a low nitrogen content. In both series of experiments the differences gave a factor of 1.6.

The experiments confirm the results, we obtained earlier from perennial field trials where an effect of manuring of the apple trees on the development of *M. ulmi* was found.

It is concluded that the mortality of predators of *M. ulmi,* caused by chemical treatment, is not the only reason why the red spider mite populations are stimulated in well kept orchards. The higher level of nitrogen content of the leaves, brought about by cultural practice, in particular manuring, may be of considerable importance also.

It is a well-known fact that in neglected orchards one can only find a very low population density of harmful mites, whereas in well-kept orchards a very severe attack of the Fruit tree red spider mite, *Metatetranychus ulmi* (Koch) often develops, with the result that a bronzing of the leaves can be observed in August or September. In many cases it has been assumed that this phenomenon must be attributed to predator elimination; in a well-kept orchard most of the predators have been killed by chemical pest control, while in the neglected one the predators still have their regulating influence.

The opinions on the effect of the predators on the mite population density differ. While it is quite obvious, that both predacious insects (particularly Mirids and Coccinellids) and predacious mites (Typhlodromids) reduce the mite population considerably (AUSTIN & MASSEE 1947, COLLYER 1949, KUENEN 1947, etc.), quantitative proof that the destruction of the predacious mites alone can account for the increase in phytophagous mites, has only recently been claimed.

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BERKER (1958) and COLLYER (1958) have succeeded in giving data which show that under the circumstances they studied the regulating effect of the predatory mite *Typhlodromus tiliae* Oudms. on the development of *M. ulmi* was very important.

In the meantime there are other cases where the relation between predators and abundance of phytophagous mites is certainly not so simple as in the cases cited above, and a further study of the causes of mite outbreaks is still very necessary. Particularly the influence of the composition of the leaves of the plant requires further study. In the literature we can find several references to this question of which the following are cited: GARMAN & KENNEDY (1949) concluded from their experiments that the population density of the two-spotted spider mite fluctuated with the vigor of the host plant (beans and peaches), due to heavy or light fertilization.

RODRIGUEZ (1951) observed under laboratory conditions a clear response of *Tetranychus bimaculatus* Harvey to a varied mineral nutrition of the tomato plant: when concentrations of all the major mineral elements in a nutrient solution were doubled, the population density of the mites was also doubled. In 1957 FRITZSCHE, WOLFFGANG & OPEL found in deficiency experiments an increase of the number of the two-spotted spider mite, *T. bimaculatus* Harvey (= *T. urticae* Koch), on *Phaseolus vulgaris* L. parallel with the increase of the total nitrogen and of the reducing sugars in the leaves. In field experiments on two varieties of *Ph. vulgaris* they also obtained the greatest mite development on the variety with the highest content of total nitrogen and reducing sugars in the leaves. HAMSTEAD & GOULD (1957) studied the effect of apple-tree vigor on the orchard mite population. In two consecutive seasons they found the number of mites of *T. urticae* and *M. ulmi* to be substantially greater in plots showing the highest nitrogen content of the leaves. KUENEN (1949) showed the influence of the condition of the host plant i.c. the apple- and plum-tree on the population density of the fruit tree red spider, *M. ulmi*. In semi-field trials egg-production proved to be much higher on trees in well-kept than in neglected orchards. KUENEN & POST (1958) mentioned the increase of the population density of *M. ulmi* in a neglected orchard after pruning and fertilizing with a nitrogen compound had been started. RODRIGUEZ (1958) grew apple-trees in nutrient culture in the greenhouse on varying levels of nitrogen and phosphorous supply. On leaves detached from these trees he cultivated *Panonychus* (*Metatetranychus*) *ulmi* (Koch) and *Tetranychus telarius* (L.). For both species there was a positive correlation with absorbed nitrogen, but this was statistically significant only for *T. telarius*. When phosphorous supply was increased absorbed nitrogen in apple foliage was negatively correlated with *T. telarius* population. It was found that in apple foliage nitrogen and phosphorous had antagonistic effects, particularly when the nitrogen supply was increased.

In our own studies (POST, 1958) we had chemical analysis made of the leaves obtained during the months of May till October from the apple varieties Schone van Boskoop and Bellefleur (standard trees, 30—50 years old) from well-kept and neglected orchards in Zeeland. This showed that there was no obvious difference in calcium-, potassium-, magnesium-, and phosphate-content of the leaves from well-kept or neglected orchards, but there was a considerably higher nitrogen-content of the leaves in well-kept orchards (see below).

Hence we suppose that the difference in mite attack between the well-kept and neglected orchards will most probably be influenced by the nitrogen-content of the leaves. To exclude as many disturbing factors as possible, a number of laboratory and semi-field experiments were set up in 1957 to investigate in more detail the influence of the nitrogen-content of the leaves.

Materials and methods

Laboratory-experiments. Discs punched from apple-leaves (diam. 18 mm) were placed on clean, moist sand in petri-dishes (Fig. 1); the moist sand keeps the leaves in good condition and the water prevents the mites from walking off the leaves. By putting the covers obliquely on the dishes the humidity in the dishes can be kept from rising too high.

The leaves were taken from an unsprayed tree, variety Bellefleur, out of a fertilized and a non-fertilized plot. The mean nitrogen percentage of the leaves for the period June-September was 2.44% and 1.68% respectively, expressed as total nitrogen of dry weight of the leaves.

In these experiments we started with larvae from the first field generation (series A), obtained from one single unsprayed tree; whenever an individual became adult it was placed on a freshly punched leaf disc; the next generation of larvae was placed on fresh leaves again and so on. Moreover we took from the third and fourth generation in the field a number of larvae from the same tree as above and started a new series of experiments (series B and C respectively). The leaves were inspected twice a day.

Semi-field trials. Under a glass-shed rootstocks E.M. XI were planted in halved oil drums (diam. 37 cm) with sand poor in humus; 7 two-years old rootstocks were planted in each drum (fig. 2). From the middle of April half of the drums were fertilized once a week with calcium nitrate, which resulted in a clear difference in the nitrogen-content of the leaves. The mean nitrogen percentage during July, August and September was 1.96 and 1.62% respectively.

Adult mites were again obtained from one single unsprayed tree and placed on the 4 topmost leaves of the young shoots of each rootstock; this region was isolated by a ring of vaseline. By regular inspection with a binocular magnifier (15 ×) the development of the mite populations could be followed quantitatively.

Results

Laboratory experiments. From Table I we see that the female mites reared on leaves with a high nitrogen-content deposited 1.6 times as many eggs per 24 hours as mites on leaves with the low nitrogen-content. Tested with the Willcoxon test the egg values were significantly higher on the fertilized trees ($p < 0.01$). Though this factor 1.6 is rather small, yet it can be of considerable importance in the course of the 5 generations which develop per year under our climatic conditions.

L. M. BREUKEL & A. POST : *Manurial treatment and mite population in orchards.*

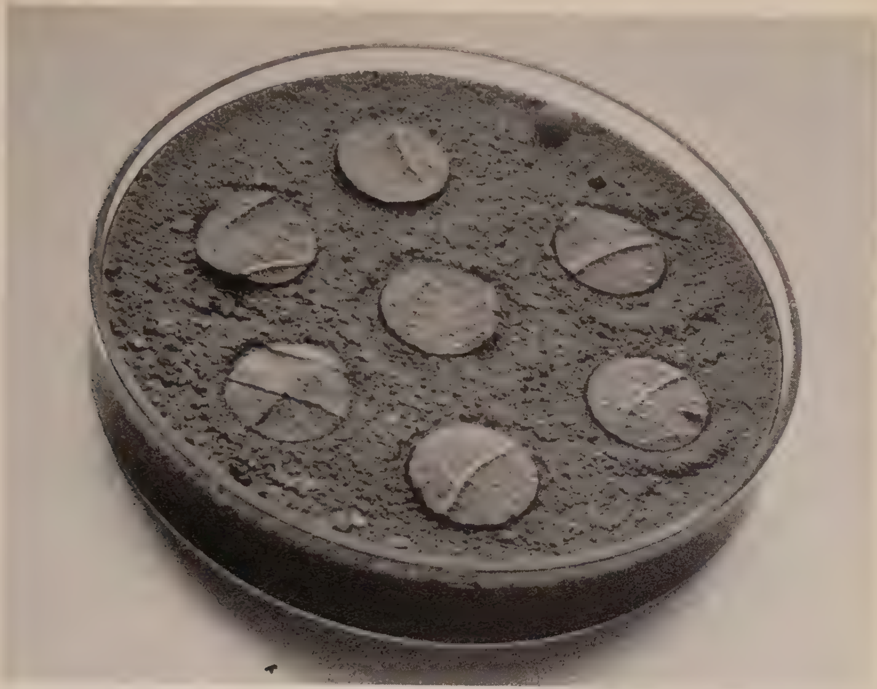

Fig. 1. Petri-dish with punched leaves on wet sand.

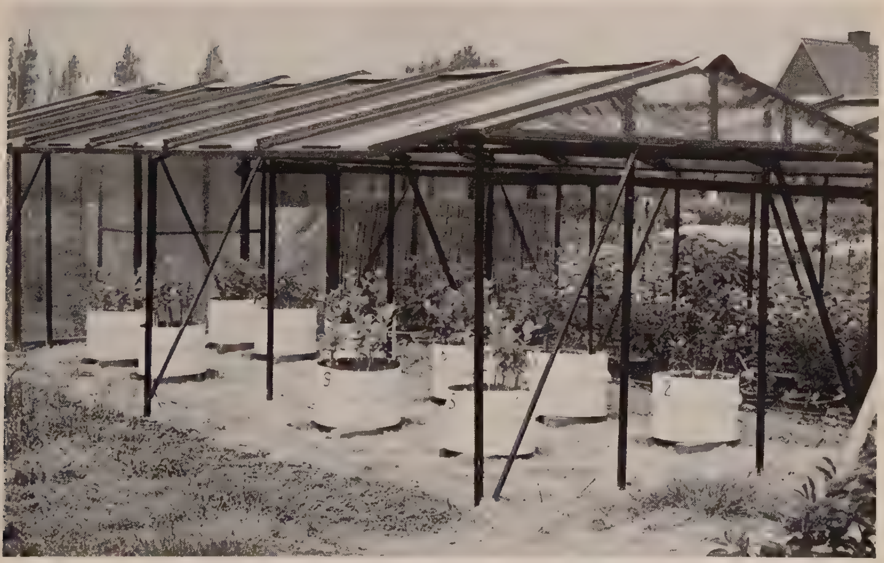

Fig. 2. Glass-shed wirth rootstocks in drums.

TABLE I

Egg-production per female in 24 hours, on leaves from fertilized and non-fertilized trees.

Fertilized trees

	A		B		C		Total	
generation	♀	eggs	♀	eggs	♀	eggs	♀	eggs
1	18	1.50 ± 0.42 1)					18	1.50 ± 0.42
2	12	1.75 ± 0.60					12	1.75 ± 0.60
3	11	1.88 ± 0.31	12	1.48 ± 0.51			23	1.67 ± 0.43
4	14	1.16 ± 0.59	12	1.14 ± 0.44	12	1.58 ± 0.56	38	1.29 ± 0.53
Total	55	1.54 ± 0.50	24	1.31 ± 0.48	12	1.58 ± 0.56	91	1.49 ± 0.50

Non-fertilized trees

	A		B		C		Total	
generation	♀	eggs	♀	eggs	♀	eggs	♀	eggs
1	15	0.87 ± 0.36					15	0.87 ± 0.36
2	14	0.88 ± 0.60					14	0.88 ± 0.60
3	8	1.03 ± 0.42	10	1.04 ± 0.39			18	1.04 ± 0.41
4	11	0.79 ± 0.25	6	0.95 ± 0.45	12	1.15 ± 0.24	29	0.97 ± 0.33
Total	48	0.89 ± 0.43	16	1.01 ± 0.42	12	1.15 ± 0.24	76	0.95 ± 0.44

1) standard deviation (σ)

TABLE II

Mortality of eggs on leaves from fertilized and non-fertilized trees.

Fertilized trees

	A		B		C		Total	
generation	number of eggs	mortality %	number of eggs	mortality %	number of eggs	mortality %	number of eggs	mortality %
1	733	11.7					733	11.7
2	192	11.9					192	11.9
3	232	9.0	187	13.9			419	11.2
4	180	3.9	82	14.6	359	6.9	621	7.1
Total	1337	10.2	269	14.1	359	6.9	1965	10.1

Non-fertilized trees

	A		B		C		Total	
generation	number of eggs	mortality %	number of eggs	mortality %	number of eggs	mortality %	number of eggs	mortality %
1	312	14.7					312	14.7
2	107	23.4					107	23.4
3	84	14.3	125	17.6			209	15.8
4	86	20.9	80	3.8	226	14.6	392	13.8
Total	589	17.0	205	12.2	226	14.6	1020	15.5

TABLE III

Mortality during development from larvae to adults, on leaves from fertilized and non-fertilized trees.

Fertilized trees

	A		B		C		Total	
generation	initial number of larvae	mortality %	initial number of larvae	mortality %	initial number of larvae	mortality %	initial number of larvae	mortality %
1	92	21.2					92	21.2
2	337	24.9					337	24.9
3	99	34.3	97	23.7			196	29.1
4	155	31.0	110	20.9	96	10.4	361	22.4
5	109	16.5	56	23.2	124	21.0	289	19.0
Total	792	25.7	263	22.4	220	15.5	1275	23.3

Non-fertilized trees

	A		B		C		Total	
generation	initial number of larvae	mortality %	initial number of larvae	mortality %	initial number of larvae	mortality %	initial number of larvae	mortality %
1	93	31.2					93	31.2
2	146	40.4					146	40.4
3	78	47.4	96	16.7			174	30.5
4	61	41.0	83	49.4	96	8.3	240	30.8
5	44	29.5	55	27.3	95	24.5	193	26.4
Total	422	38.6	234	30.8	190	16.3	846	31.4

There was no significant difference between the length of the oviposition periods of the mites on the high and low nitrogen leaves.

The mortality of eggs, larvae and nymphs on the fertilized trees proved to be significantly smaller than on the non-fertilized trees (Tables II and III): $p = 0.023$ and 0.037 respectively.

TABLE IV

Duration of development from larvae to adults on leaves from fertilized and non-fertilized trees.

		Fertilized trees			*Non-fertilized trees*		
generation	sex	number of adults	range in days	mean in days	number of adults	range in days	mean in days
1—4	♀	407	5 —12½	7.95	263	6 —14	8.76
	♂	347	4 —12	7.14	177	4½—13	7.96
5	♀	136	6½—15½	11.01	84	6 —18½	12.00
	♂	98	8 —14	9.95	58	8½—15½	11.34
Total	♀	543	5 —15½	8.71	347	6 —18½	9.55
	♂	445	4 —14	7.76	235	4½—15½	8.80

The duration of development from larva to adult of the male mite appeared to be about one day shorter than of the female mite on the fertilized as well as on the non-fertilized trees. This agrees with the well-known field observations where the male awaits the hatching of the female mite from deuterocrysalis.

The development of the male as well as the female mite on the high nitrogen leaves however is more rapid than on the low nitrogen leaves, as will be seen in Table IV and Fig. 3. This difference of about one day will not be of such importance as the difference in egg production.

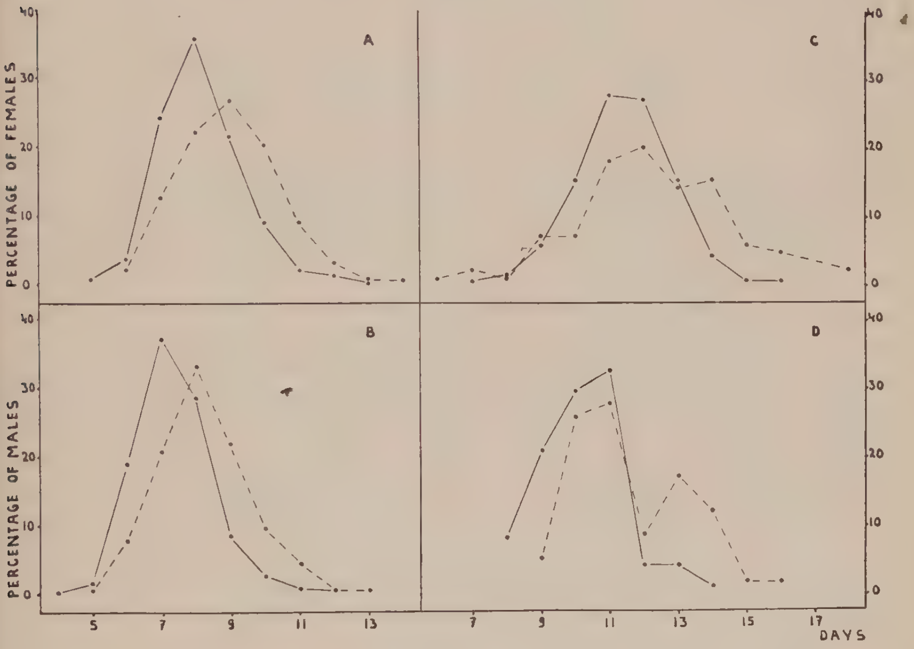

Fig. 3. Duration of development of *M. ulmi* on fertilized and non-fertilized leaves.
A & C females . ———— . high nitrogen leaves
B & D males . - - - - - - - . low nitrogen leaves
A & B generation 1—4
C & D generation 5

Semi-field trials. On the leaves of the fertilized rootstocks there is an obviously better development of the mite population (Figs. 4, 5, 6 and 7); one of the causes of this difference is the higher egg-production of the mites on these twigs (Table V).

From this table we learn that in these outdoor-experiments the reproduction-rate of the mite on the high nitrogen-content leaves is 1.6—1.7 times that on the low nitrogen-content leaves, just the same ratio as in the laboratory experiments.

As in this experiment the leaves have been inspected only once in two to four

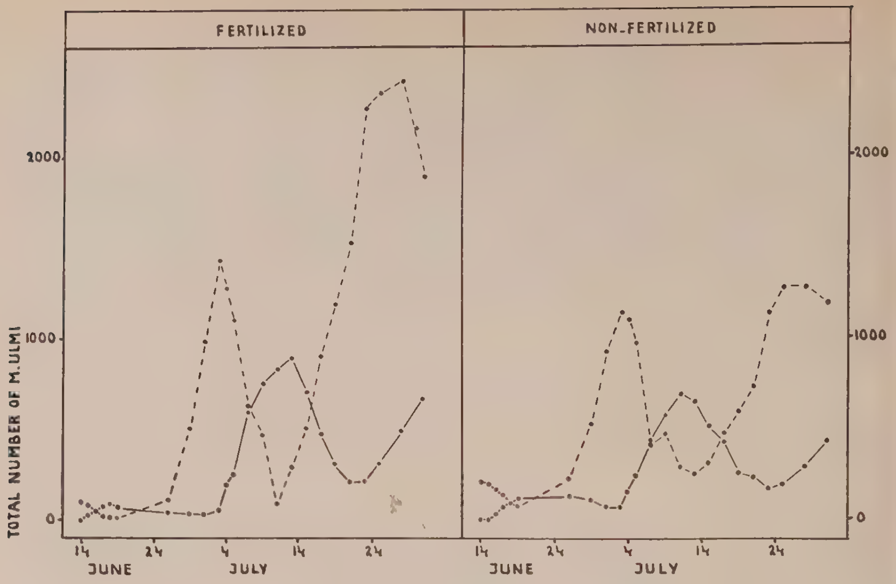

Fig. 4. Development of *M. ulmi* on fertilized and non-fertilized rootstocks.
. ———— . mites, all stages ; . - - - - - - - - - - . eggs
first period, series A.

days, small differences in period of oviposition and development from larva to adult can not be observed. In fig. 4, 5, 6 and 7 the peaks of the curves in the two series of experiments are on about the same dates. In experiments in the first

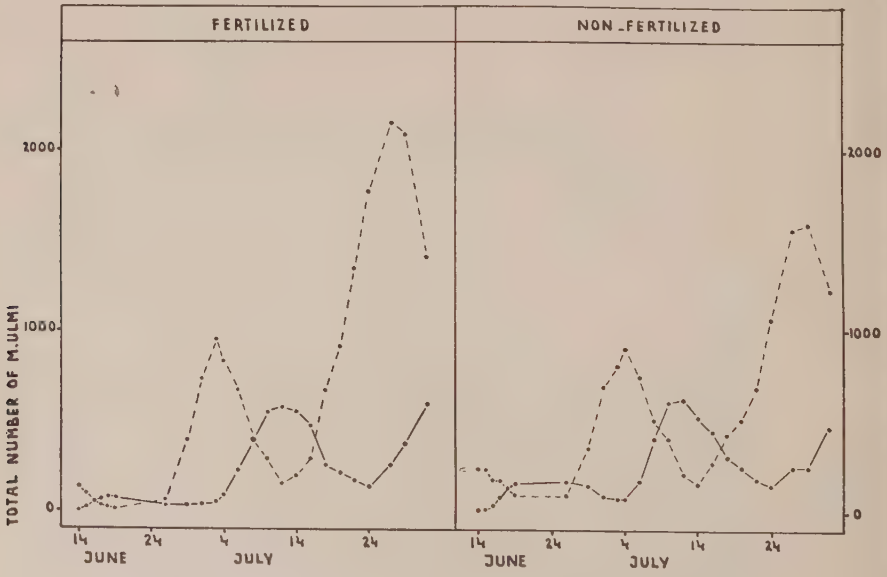

Fig. 5. Development of *M. ulmi* on fertilized and non-fertilized rootstocks.
. ———— . mites, all stages ; . - - - - - - - - - - . eggs
first period, series B.

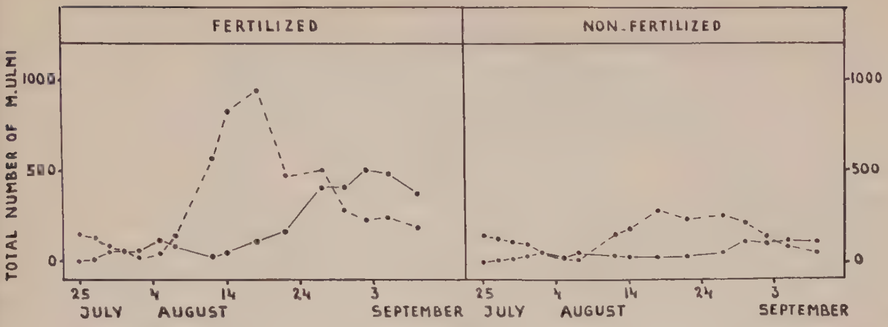

Fig. 6. Development of *M. ulmi* on fertilized and non-fertilized rootstocks.
. ———— . mites, all stages ; . - - - - - - - - - - . eggs
second period, series A.

period during June and July the population reached a higher density than in the experiments in the second period during July and August. In accordance with this we observed the maximum day-temperature in the first period to be higher than in the second period.

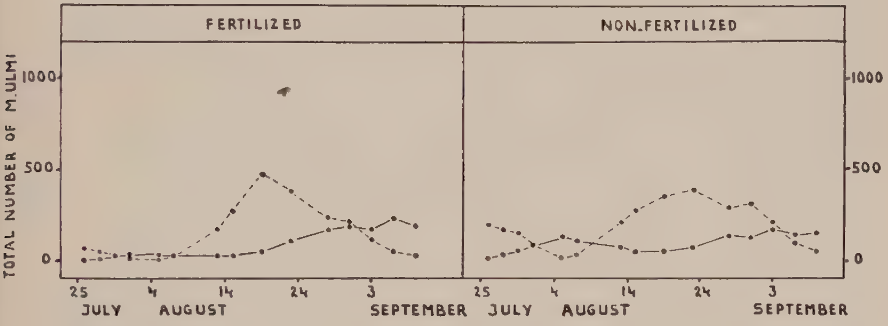

Fig. 7. Development of *M. ulmi* on fertilized and non-fertilized rootstocks.
. ———— . mites, all stages ; . - - - - - - - - - - . eggs
second period, series B.

Discussion

The results of the experiments, mentioned above, confirm the observations made in perennial field trials (Kuenen & Post 1958; Post 1958, 1959): starting with a neglected orchard with a very low population density of *M. ulmi*, we saw the population density increase after pruning and manuring with ammonium sulphate, without any pest control being performed in these plots. Concurrently we found an increasing nitrogen content in the leaves. On the other hand, in a well kept orchard with a rather high population density of *M. ulmi*, we found the density decrease in the second and third year, after pruning and manuring were stopped. In this case a decreasing nitrogen content of the leaves

TABLE V

Egg-female ratio on fertilized and non-fertilized rootstocks.

		Fertilized rootstocks			*Non-fertilized rootstocks*			
	I A	♀	eggs	ratio	♀	eggs	ratio	factor
Total of females		68	2909	42.8	127	2966	23.4	
from 26/6—5/7		59	2215	37.8	40	1076	26.9	
Total of eggs		24	912	38.0	52	1031	19.8	
from 29/6—7/7								
		151	6036	40.0	219	5073	23.3	1,7
Total of females	I B	27	914	33.8	75	1494	19.9	
from 26/6—6/7		51	1943	38.1	74	1499	20.3	
Total of eggs		54	1216	22.5	45	1012	22.5	
from 29/6—8/7								
		132	4073	30.9	194	4005	20.6	1.5
first period		283	10109	35.7	413	9078	22.0	1.6
Total of females								
from 5/8—22/8	II A	109	1982	18.2	43	429	10.0	
Total of eggs		84	1493	17.8	57	690	12.1	
from 7/8—27/8								
		193	3475	18.0	100	1119	11,2	1,6
Total of females								
from 5/8—23/8	II B	17	344	22.4	61	521	8.7	
Total of eggs		75	1203	16.0	98	1019	10.4	
from 7/8—27/8								
		92	1547	16.8	159	1540	9,7	1,7
second period		285	5022	17.6	259	2659	10.3	1.7

was also found. It is quite obvious that the mortality of predators of *M. ulmi*, caused by the chemical control, cannot be the only factor which increases the population density in well kept orchards and which may have disastrous consequences. The rise of the nitrogen content of the leaves, brought about by cultural practice, in particular manuring the trees, may be of considerable importance also.

ZUSAMMENFASSUNG

Der Einfluß der Düngung in Obstgärten auf die Populationsdichte von Metatetranychus ulmi *(Koch).*

Der Einfluß des Stickstoffgehaltes der Blätter auf die Entwicklung der Roten Obstbaum-Spinnmilbe, *Metatetranychus ulmi* (Koch), wurde unter Laboratoriums- und halbfeldmäßigen Bedingungen geprüft. Steigender Stickstoffgehalt ergibt eine höhere Eiproduktion pro Weibchen und je 24 Stunden, eine geringere Absterberate der Eier, Larven und Nymphen und eine höhere Entwicklungsgeschwindigkeit sowohl der Männchen wie der Weibchen. Im Falle der Eiproduktion und der Mortalitätsraten waren die Unterschiede gesichert. Unter den Halb-Freilandbedingungen wurde es deutlich, daß das Ei-Weibchen-Verhältnis bei hohem Stickstoffgehalt der Blätter gedüngter Wurzelstöcke ebenfalls signifikant höher war als auf

Blättern mit niedrigem N-Gehalt. Sowohl im Labor wie im Freien betrug der Faktor 1,6. Diese Feststellungen bestätigen die Ergebnisse langfristiger Feldversuche POST's über die Wirkung der Düngung von Apfelbäumen auf die Entwicklung von *Metatetranychus ulmi*. Aus diesen Befunden ergibt sich ganz deutlich, daß die Abtötung der Feinde von *M. ulmi* nicht der einzige kausale Faktor für die Zunahme dieser Milbe ist. Der Anstieg des Stickstoffgehaltes der Blätter, der durch die Kulturmaßnahmen, besonders die Düngung, hervorgerufen wird, kann ebenfalls von beträchtlicher Beteutung sein.

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ORGANOPHOSPHATE RESISTANCE AND ESTERASE ACTIVITY IN HOUSEFLIES

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Esterase activities were measured in homogenates of organophosphate resistant and susceptible houseflies using several substrates. No differences were found between the cholinesterase activities. The ali-esterase activity, however, proved to be very much lower in the resistant than in the susceptible strains. It is concluded that resistance and low ali-esterase activity are physiologically connected.

The insecticidal action of organophosphorus compounds is generally thought to be due to the inhibition of cholinesterase activity (SPENCER & O'BRIEN, 1957). Some observations on *in vivo* inhibition of esterases in the housefly (VAN ASPEREN, 1957, 1958), however, drew attention to the possible role of other esterases. A more detailed study of the occurrence and the properties of esterases in the adult housefly was therefore undertaken. The results obtained with an insecticide-susceptible strain of flies will be published elsewhere (VAN ASPEREN, 1959). The present paper deals with further investigations, in which a comparison was made between susceptible and organophosphate resistant strains of houseflies. Such a study, it was hoped, might give useful information both on the part played by the esterases in insecticidal action and on the biochemical mechanism responsible for resistance.

In some preliminary experiments the esterase activity to methylbutyrate was measured in whole fly homogenates of one susceptible and three organophosphate resistant strains. Quite unexpectedly it proved to be much lower in the resistant than in the susceptible strain. The correlation between high resistance and low ali-esterase activity, however, could have been merely incidental. The investigations were, therefore, extended to five other organophosphate-susceptible and one other organophosphate resistant strain. Moreover, the esterase activity was measured in flies obtained by crossing a susceptible and a resistant strain and reselection of the offspring by an organophosphate for several generations. Since the choice of methylbutyrate as a substrate was arbitrary, further experiments were performed using other substrates. These could possibly also yield information on the substrate preferences of the enzymes concerned. It was therefore firmly established that the ali-esterase activity in all organophosphate-resistant strains was considerably lower than in the susceptible strains. Further investigations dealt with the problem whether this low activity was due to the small amount of enzyme or to other factors, such as low enzyme-substrate affinity, the presence of inhibitors or the absence of activating factors.

The sensitivity of the ali-esterases to inhibition by organophosphates, a property which is of paramount importance for the present subject, was studied in one susceptible and three resistant strains.

DESCRIPTION OF THE INSECT MATERIAL USED

The experiments were done with various strains of *Musca domestica* L. The flies were reared on a milkpowder-yeast diet at 25° C and circa 70% relative humidity. Some data on origin, selection and resistance are shown in Table I.

TABLE I

History and properties of the strains used

strain	origin	selection in the field		selection in the laboratory	resistance	
		chlorinated hydrocarbons	organo phosphates		chlorinated hydrocarbons	organo phosphates
S	?	—	—	—	—	—*
R_{II}	Denmark	+	—	lindane	+	—
U	Uruguay	+	—	—	—	—
U_{II}	Selected from U	+	—	lindane	+	—*
MK	Netherlands	DDT	—	—	—	—
B	Netherlands	—	—	DDT	+	—
D	Denmark	+	parathion	parathion	—	+*
A	Denmark	+	diazinon	diazinon	+	+*
F	Denmark	+	diazinon	diazinon	+	+*
C	Italy	?	diazinon	diazinon	+	+*

* see fig. 1.

The strains D, A, F and C had developed resistance to organophosphorus insecticides in the field. In the laboratory, they were kept under low selection pressure of the appropriate organophosphorus compound. The resistance to some organophosphates can be derived from the LD_{50} values plotted in fig. 1. These were obtained by a contact method, in which the flies were exposed for 24 hours to a residue of the insecticide in a small amount of peanut oil on the walls of small glass jars.

The strains S, R_{II}, U, U_{II}, MK and B were collected in the field before the application of organophosphorus compounds and were not selected with organophosphates in the laboratory. Nevertheless two of them (R_{II} and U_{II}) showed some resistance to these compounds, though clearly less than the organophosphate selected strains (see fig. 1).

In both groups some of the strains were more or less resistant to chlorinated hydrocarbons as a result of selection in the field and/or in the laboratory. When, however, in this paper the terms resistant and susceptible are used they will refer to the organophosphorus insecticides. All selections were carried out by exposing the flies overnight to filter papers impregnated with the insecticides.

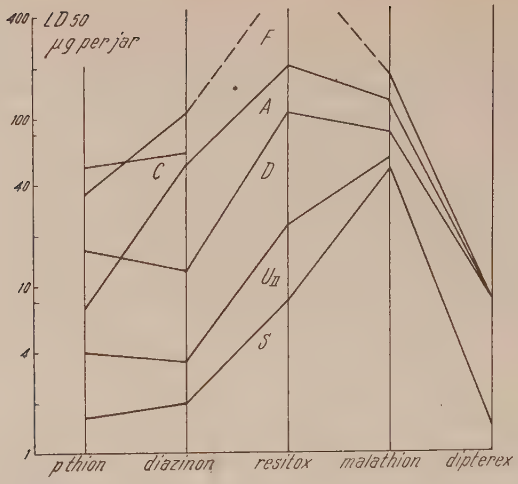

Fig. 1. Susceptibility to organophosphates.
Resitox = O,O-diethyl O-3-chloro-4-methyl-umbelliferone thionophosphate.
Dipterex = O,O-dimethyl 1-hydroxy-2-trichloroethyl phosphonate.

Crossing experiment. The ali-esterase activity was also measured in "Cr-flies" obtained in the following crossing-experiment:

$$F \times S \rightarrow f_1 \rightarrow f_2 \rightarrow f_3$$
$$f_3 \times S \rightarrow f_1 \rightarrow f_2 \rightarrow f_3 \rightarrow f_4$$
$$f_4 \times S \rightarrow f_1 \rightarrow f_2 \rightarrow f_3 \rightarrow f_4 \text{ (Cr)}$$

The f_2, f_3 and f_4 generations were selected by exposing the males and females separately to filterpapers impregnated with diazinon giving about 80% mortality. The reciprocal crossings were carried out, but since no difference was found in the resistance of their offspring, the flies of both crosses were not kept apart in the selections.

DESCRIPTION OF THE METHOD USED FOR DETERMINING ESTERASE ACTIVITY

Measurement of hydrolytic rates was performed by the Warburg manometric method at 27°C in a total volume of 2.2 ml of solution containing 0.14 M NaCl, 0.025 M $NaHCO_3$ and fly tissue, substrate and inhibitor at the concentrations required; the gas phase contained 95% N_2 and 5% CO_2, pH was about 7.5. After 5 minutes temperature equilibration readings were taken at 10 min. intervals for one hour. In the experiments on inhibition of esterase activity by paraoxon the homogenate and the inhibitor were incubated for one hour at 27°C. Incubation took place under the same experimental conditions as measurement of hydrolysis, except for the absence of substrate. In many experiments 10^{-5} M eserine was added in order to inactivate the cholinesterase. This does not affect the ali-esterase activity (VAN ASPEREN, 1959).

Homogenates. As it had been found that ali-esterases are extremely sensitive to heating in slightly alkaline media (VAN ASPEREN, 1959), care was taken to

Esterase activities in different strains of flies.

mean = mean activity, expressed in μl CO_2/30 min./fly; number of estimations in brackets; max. dev. = maximal deviation from the mean; perc. = activity expressed as a percentage of the activity in strain S.; ratio = mean act. susceptible strains/mean act. in resistant strains.

In the experiments with phenylacetate, phenylpropionate, phenylbutyrate, amylacetate and triacetin as substrates eserine was added in 10^{-5} M conc. in order to eliminate the hydrolytic activity of the cholinesterase present (see VAN ASPEREN 1959).

			Organophosphate susceptible				Organophosphate resistant			
			S	R	U	U_{II}	D	A	F	ratio
Acetylcholine 0.015 m	♂	mean	76(1)	76(1)	72(1)	78(1)	76(1)	81(1)	92(1)	
		perc.	100%	100%	95%	103%	100%	107%	121%	0.9
Amylacetate emulsion	♂	mean	26(2)	26(2)	23(2)	27(2)	23(2)	22(2)	30(2)	
		max. dev.	3	1	0	0	2	3	1	
		perc.	100%	100%	88%	104%	88%	85%	115%	1.0
Triacetin 0.05 M	♂	mean	35(2)	34(2)	29(2)	38(2)	22(2)	28(2)	31(2)	
		max. dev.	4	3	1	4	3	1	3	
		perc.	100%	97%	83%	109%	63%	80%	89%	1.3
Tributyrin emulsion	♀	mean	88(1)	133(1)	142(1)	136(1)	50(1)	73(1)	74(1)	
		perc.	100%	151%	161%	155%	57%	83%	84%	1.9
	♂	mean	95(1)	110(1)	117(1)	114(1)	45(1)	60(1)	67(1)	
		perc.	100%	116%	123%	120%	47%	63%	71%	1.9
Phenylacetate emulsion or 0.009 M	♂	mean	143(5)	123(5)	125(5)	152(5)	40(6)	58(5)	60(5)	
		max. dev.	18	37	41	27	24	11	9	
		perc.	100%	86%	87%	106%	28%	41%	42%	2.6
Phenylpropionate emulsion	♂	mean	219(2)	270(2)	238(2)	268(2)	72(3)	118(2)	106(2)	
		max. dev.	25	3	7	11	8	7	8	
		perc.	100%	123%	109%	122%	33%	54%	48%	2.5
Phenylbutyrate emulsion	♂	mean	297(2)	307(2)	303(1)	377(2)	65(3)	100(2)	114(2)	
		max. dev.	31	25	—	18	12	25	16	
		perc.	100%	103%	102%	127%	22%	34%	38%	3.5
Ethylbutyrate emulsion	♂	mean	281(1)	282(1)	278(1)	302(1)	54(1)	39(1)	55(1)	
		perc.	100%	100%	99%	107%	19%	14%	20%	5.8
Methylbutyrate 0.048 M	♀	mean	383(3)	312(1)	410(1)	362(2)	68(2)	119(2)	77(2)	
		max. dev.	20	—	—	3	12	9	5	
		perc.	100%	81%	107%	95%	18%	31%	20%	4.2
	♂	mean	284(13)	247(1)	316(1)	332(1)	40(10)	66(5)	52(5)	
		max. dev.	71	—	—	—	20	27	6	
		perc.	100%	87%	111%	117%	14%	23%	18%	5.7

The esterase activity to methylbutyrate in MK ♀♀ and B ♂♂ was found to be 343(90%) and 335 (87%) respectively

The esterase activity to methylbutyrate in C ♂♂ was found to be 26 (9%).

minimize exposure of the homogenates to heat and alkali. The flies were homogenized, therefore, in ice-cold 1% NaCl, using a small type Waring blendor. The flasks containing the homogenates were kept in ice-water till use. Sodium bicarbonate was added at the very last moment before incubation or measurement started, thus ensuring the pH of the homogenate not to rise above 7.5.

Substrates. Several commercially obtained esters of acetic acid, propionic acid and butyric acid were used as substrates. They are listed in Table II. Many of these are badly soluble in water and could be used as emulsions only. These were prepared by homogenizing 1 ml of ester with 0.2 ml of an emulsifier (Atlas Powder Co, G 7076 H) in 40 ml salt solution; 0.8 ml of emulsion was added per Warburg flask.

EXPERIMENTAL RESULTS

Comparison of esterase activity. Table II shows the results of esterase activity measurements, in which 7 strains of houseflies and 9 substrates were used. The data obtained with methylbutyrate in three other strains are given at the bottom of this table. The results may be summarized as follows:

1. The cholinesterase activities (substrate acetylcholine) in homogenates of organophosphate-susceptible and organophosphate-resistant flies are approximately equal. This finding has been confirmed in several other experiments (see also OPPENOORTH, 1958).
2. The ali-esterase activities measured with amylacetate or triacetin as substrates (in the presence of eserine) in susceptible and resistant strains do not differ very much. With both substrates the ratio (activity susceptible strains/activity resistant strains) is close to unity.
3. Large differences between susceptible and resistant strains can be observed in the ali-esterase activities to tributyrin, phenylacetate, phenylpropionate, phenylbutyrate, ethylbutyrate or methylbutyrate. The ratio varies from approximately 2 in the case of tributyrin to nearly 6 in the case of methyl- and ethylbutyrate.

Influence of methylbutyrate concentration. The lower ali-esterase activities in the resistant flies need not necessarily be caused by a smaller amount of enzyme. They could also be due to a lower affinity of the enzyme for its substrates. This possibility was investigated in an experiment with the strains S (susceptible) and D (resistant) using methylbutyrate as a substrate. The rate of hydrolysis was measured at two methylbutyrate concentrations, viz. that used in the experiments listed in Table II and one more than twice as high. The results are given in Table III.

TABLE III

Esterase-activity to methylbutyrate at two substrate-concentrations expressed as μl CO_2/30 min./ fly.

	Methylbutyrate-conc.	
	0.048 M	0.102 M
Strain S	258	360
Strain D	42	43

It is clear that raising the substrate concentration does not reduce the difference as would be expected if low affinity of the enzyme in the resistant flies for its substrate was the cause of the difference.

The possible influence of inhibitors or activators. This possibility was studied *a.* by comparing the activities of different homogenate concentrations, and *b.* by mixing homogenates of resistant and susceptible flies. Table IV shows the results of an experiment, in which the strains S and D were used with methylbutyrate as substrate.

TABLE IV

Inhibiting or activating factors

Activities are expressed as µl CO_2/30 min./Warburg flask; The numbers of flies per flask used in each determination can be found in the first horizontal column (D) and in the first vertical column (S) of the table.

		Strain D			
		—	1 fly	2 flies	4 flies
	—	—	56	120	248
Strain S	½ fly	212	249	—	—
	1 fly	379	430	—	—

The concentration of the homogenates does not appreciably alter the activity per fly as would be expected if activating or inhibiting factors were involved. In mixtures of S and D the activities of both components are additive: there are no indications either that the activity in S is decreased by some inhibitor present in D or that the activity in D is increased by some activating factor present in S.

Inhibition by paraoxon. It has been found (VAN ASPEREN, 1959) that in susceptible fly homogenates the esterase activity to methylbutyrate is strongly inhibited by paraoxon. In the present study some experiments were done in which the sensitivity of methylbutyrate hydrolysis to paraoxon was measured in homogenates of both susceptible and resistant strains.

In accordance with our earlier findings the I_{50} (conc. of paraoxon giving 50% inhibition) for homogenates containing one susceptible fly (S) per flask, proved to be approximately 3×10^{-9} M. Inhibition measurements with homogenates of resistant flies (D, A, F) were quantitatively less reliable as a consequence of the much lower normal activities. Nevertheless, the available data allow of the conclusion, that at low concentrations the inhibition was roughly similar to that obtained with the susceptible strain. At higher concentrations, however, the percentage inhibition was distinctly lower in the resistant fly homogenates and appeared not to surpass a certain level. Further experiments, therefore, dealt with the inhibition of the esterase activity to methylbutyrate by high concentrations of paraoxon. Table V shows the results obtained with homogenates of 4 different strains.

The activities remaining after addition of 1.2×10^{-7} M paraoxon are strikingly similar. However, in the resistant strains D, A and F the remaining activity averages 34% of the normal activity (without inhibition), whereas it is only 4%

TABLE V

Inhibition of methylbutyrate hydrolysis by paraoxon.

The table lists the activities, expressed as µl CO_2/30 min./Warburg flask, together with the percentage of the normal activity remaining after inhibition. Each flask contains 4 flies.

paraoxon-concentration	Strain S		D		A		F	
0	1186*		149		188		167	
1.2×10^{-7}	46	4%	58	39%	53	28%	58	35%
1.2×10^{-5}	38	3%	29	19%	25	13%	40	24%

* This value was obtained by taking 4 times the activity measured with 1 fly/flask.

of the normal activity in strain S. A similar picture was obtained after addition of 1.2×10^{-5} M paraoxon. These data suggest that an enzyme which is rather insensitive to paraoxon and which is present in about equal amounts in the susceptible and the resistant strains is responsible for part of the hydrolysis of methylbutyrate by breis of houseflies.

The crossing experiment. Crossing the resistant strain F with the susceptible strain S, followed by repeated backcrossing of the offspring with strain S, will gradually remove the genetic material originating from strain F. Removal of genes responsible for resistance to diazinon will, however, be counterbalanced by the concentrating effect of the selection. This, of course, also applies to factors which are genetically linked with the resistance. The Cr-flies finally obtained may, therefore be expected to show only those properties of strain F which are physiologically connected or genetically linked with diazinon-resistance. Table VI compares the data on susceptibility to diazinon and esterase activity to methylbutyrate for S, F and Cr.

TABLE VI

Susceptibility and ali-esterase activity of the flies obtained in the crossing experiment.

LD50 for diazinon is expressed as µg of diazinon per jar. Ali-esterase activity is expressed as µl CO_2/30 min./fly; substrate methylbutyrate 0.048 M.

Strain	S	Cr	F
LD50 diazinon	1.5	80	150
Ali-esterase activity	284	99	52

It is evident that the Cr-flies are highly resistant to diazinon, though not as resistant as those of strain F, and also that their esterase activity is very low, though not as low as that of strain F. It seems justified, therefore, to conclude that the low ali-esterase activity is connected, either physiologically or genetically, with the resistance to diazinon.

DISCUSSION

The most striking observation made in the course of the present investigations was the extremely low ali-esterase activity to a number of substrates in the organophosphate resistant strains as compared to that in the susceptible strains. Before dealing with this remarkable difference some other topics will be discussed.

1. The mode of action of organophosphorus compounds. It has been found that the ali-esterase activity to methylbutyrate in susceptible houseflies at the time of knockdown due to DDVP-poisoning is more strongly inhibited than the cholinesterase activity (83% and 27% respectively; v. ASPEREN, 1958). This observation suggested the possibility that organophosphate poisoning might be due to ali-esterase inhibition. Resistance could then have been acquired by an increase of the amount or activity of the ali-esterase involved or by a decrease of the sensitivity of this enzyme to organophosphates. The present results do certainly not support this suggestion and it must, therefore, be considered less probable that an ali-esterase is the primary site of attack in organophosphate poisoning. The clear-cut correlation between the level of ali-esterase activity and the susceptibility to organophosphorus compounds, however, strongly indicates that ali-esterases are in some way involved in the processes that influence the course of the intoxication by organophosphates.

2. The number of esterases involved. In a previous paper we discussed the occurrence and substrate specificity of the esterases in susceptible houseflies (VAN ASPEREN, 1959). It was postulated there that at least two different esterases were concerned with the hydrolysis of the substrates studied, viz. a cholinesterase, splitting choline esters and acetic acid esters but not acting on butyric acid esters, and an ali-esterase hydrolysing esters of butyric acid and acetic acid but showing no activity to choline esters. The former was found to be strongly inhibited by eserine, the latter proved to be insensitive to this compound. Evidence has now accumulated that there are at least two and most probably more ali-esterases present in houseflies. Part of this evidence is included in this paper.

Table II shows that the ratio of the ali-esterase activities in susceptible and resistant flies is highly dependent on the substrate used. Its value varies from 1.0 for amylacetate to 5.8 for ethylbutyrate. This can most easily be explained by assuming that at least two ali-esterases are involved, one being present in approximately equal amounts in susceptible and resistant flies and another being present in much lower amounts in the resistant than in the susceptible flies. The extent to which each of these two ali-esterases contributes to the hydrolysis of a given substrate will then determine the value of the ratio. If the two enzymes involved have different affinities for the substrate under investigation, the ratio obtained will be strongly dependent on the substrate concentration used. This has not been investigated any further. It is obvious, however, that the value of the ratio given in Table II should be very cautiously interpreted. Alternative explanations for the widely divergent ratios are possible. Unless activating or inhibiting factors acting in the hydrolysis of some and not in the hydrolysis of other substrates are postulated, these explanations will all have to include the existence of different ali-esterases. Since an influence of activating or inhibiting factors in the case of methylbutyrate hydrolysis is highly improbable (Table IV), it seems justified to conclude that at least two different ali-esterases are involved in the hydrolytic processes studied.

Further evidence in favour of this view may be derived from the results of the experiments on paraoxon inhibition (see Table V). These seem to indicate the presence of two ali-esterases acting on methylbutyrate, one being very sensitive to paraoxon whereas the other is inhibited by high concentrations of paraoxon only. The latter enzyme, which is responsible for only 4% of the total hydrolytic activity to methylbutyrate in the susceptible flies but for about 34% in the resistant flies, shows a ratio-value (S / D, A,F) close to unity. This should not be interpreted to mean that the paraoxon-insensitive enzyme splitting methylbutyrate is identical with the ali-esterase hydrolysing amylacetate or triacetin: there may be several enzymes with approximately equal activities in susceptible and resistant flies.

3. The relation between ali-esterase activity and resistance. Four independently selected resistant strains[1]) all showed a strikingly low ali-esterase activity, whereas the activity in five independently collected susceptible strains was found to be high. There can be little doubt, therefore, that selection for resistance involves selection for low ali-esterase activity. This proves the identity of the genes for low activity with genes for resistance, which implies a physiological connection between the two properties. The results of the crossing experiment are in full accordance with this view.

With respect to the nature of the physiological connection two alternatives can be mentioned: either the low ali-esterase activity is the cause of resistance or the two properties are caused by some common factor. Since the physiological function of the ali-esterases in the fly is unknown it is as yet impossible to discriminate between the two alternatives.

From the data on susceptibility (Fig. 1) and those on ali-esterase activity (Table II) it can be concluded that there is no proportionality between these two properties. This points to the occurrence of factors of resistance not associated with low ali-esterase activity, which add to or modify the resistance that is associated with low ali-esterase activity. Such factors must also be held responsible for the differences in susceptibility between the susceptible strains (e.g. S and U_{II} in fig. 1.). A further argument for the occurrence of several resistance factors can be derived from the differences between the cross-resistance patterns of the strains studied. So far all strains showing an appreciable degree of organophosphate resistance as a result of selection with organophosphorus insecticides in the field have been found to contain the factor associated with low ali-esterase activity. Though this does not prove that this factor is a necessary condition for resistance, it underlines its importance.

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[1]) After the manuscript had been finished a strain was obtained from Dr. K. D. QUARTERMAN and Dr. R. W. FAY, Savannah, showing high malathion resistance and a low resistance to parathion and diazinon. Just as in the other phosphate resistant strains the esterase activity to methylbutyrate proved to be low (72 μl CO_2/30 min./fly).

ZUSAMMENFASSUNG

Die Cholinesterase- und Aliesterase-Aktivität in Homogenaten von fünf Phosphoresterresistenten und sechs Phosphorester-empfindlichen Stubenfliegen-Stämmen wurde mit der Warburg-manometrischen Methode gegenüber mehreren Substraten gemessen. Weitere Untersuchungen beschäftigten sich mit der Natur der gefundenene Unterschiede. Die Resultate (Tabelle II) können in folgender Weise zusammengefaßt werden:

1. Resistente und empfindliche Stämme zeigen eine ungefähr gleiche Aktivität der Cholinesterase.

2. Die Aliesterase-Aktivität ist gegenüber mehreren Substraten in den resistenten Stämmen sehr viel niedriger als in den empfindlichen.

3. Die niedrige Aliesterase-Aktivität der mit Phosphorester selektierten Stämme ist physiologisch mit der Resistenz verbunden. Die Natur dieses Zusammenhangs ist unbekannt.

4. Es ist unwahrscheinlich, daß Aliesterasen einen primären Angriffspunkt in der Phosphorester-Vergiftung darstellen.

5. Das Verhältnis der Aktivitäten (empfindlich/resistent) wechselt sehr stark vom einen zum anderen Substrat. Daraus wird geschlossen, daß mehrere Aliesterasen in dem untersuchten Prozeß wirksam sind.

6. Wenn Methylbutyrat als Substrat benutzt wird, ist das Verhältnis etwa fünf zu eins. Dieses hohe Verhältnis kann nicht auf Unterschiede in der Enzym-Substrat-Affinität (Tabelle III) oder auf die Wirkung aktivierender oder hemmender Faktoren zurückgeführt werden (Tabelle IV). Höchstwahrscheinlich besteht also ein Unterschied in der Menge des Enzyms (oder in der Umsatz-Zahl des Enzyms).

7. Sowohl die resistenten als auch die empfindlichen Stämme enthalten eine Paraoxonunempfindliche Aliesterase (Tabelle V).

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PATTERNS OF INSECTICIDE RESISTANCE TO ORGANO-PHOSPHORUS COMPOUNDS IN STRAINS OF HOUSEFLIES FROM VARIOUS SOURCES

BY

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Four strains of housefly (*Musca domestica* L.), resistant to organo-phosphorus insecticides, have been maintained in laboratory culture, together with a normal colony. Resistance in one strain had been developed by laboratory selection and in the others by field usage of insecticides followed by laboratory selection. They originated in Denmark, Italy and U.S.A., mainly by the use of parathion, diazinon and malathion respectively. The "resistance spectra" of these strains were determined by measuring their relative resistance to 14 organo-phosphorus compounds, by a microdrop application test. The strains selected by parathion and diazinon gave rather similar resistant patterns, showing highest resistance to parathion, paraoxon and diazinon and low resistance to malathion. The American strain produced a different pattern with high resistance to malathion and low resistance to parathion, paraoxon and diazinon. It is believed that these two patterns reflect different resistance mechanisms.

A detailed comparison of the two European strains revealed more divergence in compounds containing $(EtO)_2P$— groups than in those with $(MeO)_2P$— groups.

Injection of parathion reduced its resistance level considerably and the resistance to paraoxon on injection was even more reduced.

One line of attack on the problem of insecticide resistance is to work out the "resistance spectrum" for each strain involved. This is done by determining the median lethal doses of a range of insecticidal compounds and expressing them as ratios of similar data for a normally susceptible colony of the same species. The primary interest of a resistance spectrum is its practical value in indicating the various insecticides involved in any given case of resistance. Generally speaking, these will fall into one of the following groups: (i) DDT analogues, or (ii) BHC plus dieldrin analogues, or (iii) organo-phosphorus compounds; but it is possible for a colony of insects to acquire double (or even treble) resistance, embracing more than one group.

A more detailed examination of a resistance spectrum can give additional information of more fundamental interest, since the pattern of resistance towards a group of related poisons presumably reflects the efficiency of the protecting mechanism in coping with varieties of a type of poison. Then, if a certain pattern is common to a number of different strains, the inference is that the same mechanism is common to all. This interpretation is supported by the observation that the characteristic pattern can be evoked by selection by any insecticide in the group and in different localities, which has occurred with the BHC-dieldrin type of resistance in *Musca domestica* L., *Anopheles gambiae* Giles, *Cimex lectularius* L. and probably, in *Culex fatigans* Wiedemann. On the contrary, where resistant strains show marked differences in their detailed resistance spectra, it seems likely that more than one defence mechanism can occur. This appears to be true of DDT-resistance in *Musca domestica* and *Aedes aegypti* L., both of which probably depend largely on enzymatic dehydrochlorination, but some strains of which seem to possess additional defences, such as increased or modified lipoid deposits.

In the hope that some information of this type might be forthcoming, the resistance spectra have been worked out for several strains of housefly, resistant to organo-phosphorus compounds.

MATERIAL AND METHODS

Strains of Houseflies

The following colonies of flies have been maintained and used in tests.

Normal. A colony which has been reared in the laboratory for a large number of years, at the London School of Hygiene and Tropical Medicine, was used for basic comparisons. It is believed to be a homogeneous, normally susceptible strain; in any case, it has not significantly changed in susceptibility to DDT during the past 10 years, nor to parathion during the past 4 years.

"DFP-Resistant". In 1954, Professor L. E. Chadwick sent me a sub-colony of a strain which he had been selecting in the laboratory for over 100 generations, by exposure of the adults to DFP vapour. My tests with this strain were done in 1954-5, but have not been published, as they did not appear to add much to the brief observations already published (CHADWICK, 1954).

Danish Resistant. This originated in the field, following the extensive use of parathion treated cloth strips on Danish farms. It was collected in 1955 (KEIDING, 1956) and maintained in the laboratory (as Strain 79b); further selection was applied by exposure to parathion in 13 generations out of 26. A sub-colony was sent to Dr. F. J. Oppenoorth, Utrecht, Netherlands (who kept it as Strain D) and he sent me a sub-colony in October, 1957.

Italian Resistant. Resistance to organo-phosphorus compounds developed in houseflies in the Latina province, following 5 years of house spraying by diazinon (SACCA, 1957). A colony of these flies was further selected and inbred in the laboratory by Dr. G. Sacca who kindly sent me some in December, 1957.

American Resistant. In the U.S.A., organo-phosphorus insecticides have been used against flies on farms and poultry farms for about 5 years, mainly in the form of poison baits. Malathion was often used as the toxic ingredient and resistance to it has been noted in Florida (LABREQUE & WILSON, 1957), in Georgia (FAY, KILPATRICK & MORRIS, 1958) and elsewhere. Some of these resistant strains have been colonised at the Communicable Disease Center, Savannah, Georgia, and Dr. R. W. Fay kindly sent me a sub-colony of one ("Anderson 45") in May, 1958. This had been further selected with malathion in the laboratory.

Maintenance

All colonies were reared in a constant temperature room at 27° C. (80° F.), in cages supplies with sugar and pads soaked in milk and water (50 : 50). They were reared on a medium made from dried milk, dried yeast and agar stirred into boiling water, as described in detail elsewhere (Busvine, 1958). The rearing cycle was about 10 days and the flies were tested about 3 days after emergence.

Previous treatment of these colonies had resulted in rather highly selected, inbred populations approaching homogeneous resistance (as shown by steep dose/kill curves). Accordingly they were normally maintained without exposure to insecticides. However, in all the resistant colonies there were 2 or 3 occasions in which some susceptible individuals were observed and these were then exterminated by exposure to the appropriate insecticide. It is possible that these susceptible flies resulted in accidental contamination or invasion of the stock cages; but it is more likely that the colonies were not genetically pure. (My colleague V. D. Nguy has been able to show that high level resistance to organo-phosphorus is probably controlled by a single dominant gene. It is therefore difficult to detect heterozygotes and exclude the last few susceptible recessive genes from a population.)

Insecticides used

Samples of the following compounds were obtained in a fairly high degree of purity: —

"Chlorthion":	dimethyl 3-chloro-4-nitrophenyl phosphorothionate
diazinon:	diethyl 6-methyl-2-isopropyl-4-pyrimidinyl phosphorothionate
"Dicapthon":	dimethyl 2-chloro-4-nitrophenyl phosphorothionate
dichlorvos (DDVP):	dimethyl 2 : 2-dichlorovinyl phosphate
dichlorvos-ethyl:	diethyl 2 : 2-dichlorovinyl phosphate
dimefox:	NNN^1N^1-tetramethylphosphorodiamidic fluoride
EPN:	ethyl *p*-nitrophenyl phenyl-phosphorothionate
malathion:	S-1 : 2-di-(ethoxycarbonyl) ethyl dimethyl phosphorothiolothionate
paraoxon:	diethyl *p*-nitrophenyl phosphate
parathion:	diethyl *p*-nitrophenyl phosphorothionate
parathion-methyl:	dimethyl *p*-nitrophenyl phosphorothionate
phorate:	diethyl S-ethylthiomethyl phosphorothionate
"Potasan":	diethyl 4-methyl-7-hydroxycoumarin phosphorothionate
thiometon:	dimethyl S-ethylthioethyl phosphorothionate

Testing Method

Batches of about 30 flies were treated by applying minute drops (0.35 mm^3) to the back of the thorax of each fly, after stupefaction with carbon dioxide. A Burroughs Wellcome "Agla" microsyringe was employed, with an attachment for simplifying repeated delivery of a standard volume (Busvine, 1956). After treatment, the flies were put (sexes separately) into cages plugged with damp cotton wool, and kept at 27° C until the next day, when mortality counts were made.

For reasons explained some years ago (Busvine, 1951) a nonvolatile carrier liquid was preferred, though many workers still use acetone. Since many organo-phosphorus compounds will not dissolve satisfactorily in mineral oil, olive oil was employed instead. This is not an ideal choice, since it is a variable mixture; and though all the tests to be reported were done with one large sample of refined oil, this might differ from samples used by other workers. Furthermore, olive oil contains components which may slowly react with certain organo-phosphorus compounds, therefore most of the solutions of insecticides were used within a short time of preparation. However, nearly all the samples dissolved easily at the rate of 1% (w/v) and dilutions were made with the aid of hypodermic syringes (Oils are too viscous to measure by pipette) 1).

TABLE I

Absolute toxicity values of various organo-phosphorus compounds to the housefly (by topical application) and the rat (by oral administration). Values for the housefly, as described in this paper and compared with published data, as follows: — (a) Hadaway & Barlow (1957); (b) Lewallen (1954); (c) Keiding (1956); (d) March & Metcalf (1949); (e) [illegible] & Mahin (1953); (f) Krueger & Casida (1957). Values for the rat, from various authors, mainly quoted by Metcalf (1955).

Compound	Acute LD_{50} (mg kg)		Compound	Acute LD_{50} (mg kg)	
	Housefly (contact)	Rat (oral)		Housefly (contact)	Rat (oral)
"Chlorthion"	20, 19^a, 16^b	1500	malathion	55, 44^a, 28^b, 30^f	1400—2000
diazinon	5.7, 4.4^a, 4.6^b, 2.0^c	220—270	paraoxon	2.0, 0.5^d, 0.5^f	2—3.5
"Dicapthon"	3.7, 0.8^d	500—1000	parathion	3.0, 1.1^c, 0.9^d	6—15
dichlorvos	1.6, 0.75^e	73	parathion-methyl	2.4, 1.0^d, 1.5^f	—
dichlorvos-ethyl	4.0, 1.8^e	—	phorate	9	—
dimefox	30	3—5	"Potasan"	18	19—25
EPN	4.0, 1.9^d	12—40	thiometon	16	—

RESULTS

Expression of the Results

The mortality counts from male and female flies were collected from a number of batches (usually from different generations) for each concentration of insecticide and the whole series expressed as percentages. The females were less susceptible throughout. The average percentage kills for the two sexes were converted to probits and plotted against the logarithm of the concentrations used. This utilization of the mean percentage of the sexes was convenient in that it produced probit values for tests in which one sex showed 0% or 100% kill and it was economical in using all the flies available. Theoretically this procedure will tend to produce flatter probit/concentration curves; but since these were reasonably steep (b ranging from 2.5 to 4.5) it evidently did not cause a serious loss of precision.

The probit/log. concentration curves, both of the susceptible and resistant

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[1]) After completion of the work described in this paper my attention was drawn to the compound dioctyl phthalate, by the secretariat of the World Health Organization. This compound seems to be preferable to olive oil for the tests described. It is a good solvent for most organo-phosphorus compounds, is harmless to the flies and in limited tests gave results similar to olive oil, which it seems to resemble in physical properties.

strains, were all apparently straight and reasonably parallel. Therefore, it was considered satisfactory to express the results simply as median lethal doses, which were estimated graphically.

Toxicity of the Insecticides

The potency of the various insecticides against normal houseflies may be judged from the median lethal doses in Table I, which also gives some indication of mammalian toxicity. It will be observed that the values I obtained are nearly all higher than those quoted by other workers (Table I). This is probably due to the use of a non-volatile solvent, which is known to give rather higher equitoxic doses (for susceptible flies) than a volatile solvent such as acetone, which was used by the other investigators. (See BUSVINE, 1951, and BARKER & RAWHY, 1957).

Apart from this, it is possible that normal fly colonies vary somewhat in their response to different poisons, as do rats or other experimental animals. In general, however, there is reasonable agreement in the order of toxicity of the various compounds.

Relative Resistance in Various Fly Strains

Median lethal doses of the various insecticides were obtained with the three resistant fly colonies in the same way, (Table II). This presented no difficulty, since the probit/log. dose regression lines were all straight and reasonably steep; and in all cases it was possible to approach 100% kill. This contrasts with certain analogous work with synthetic chlorinated insecticides, where extremely high levels of resistance sometimes produce almost complete tolerance.

TABLE II

Median lethal doses of organo-phosphorus compounds to various strains of housefly

Compound	LD_{50} (μg per fly) for various strains				
	Normal	"DFP"	Danish	Italian	American
"Chlorthion"	0.35	0.56	2.8	2.8	2.5
diazinon	0.10	0.31	0.63	2.3	0.16
"Dicapthon"	0.066	0.15	0.45	1.5	0.87
dichlorvos	0.028	0.070	0.070	0.084	0.049
dichlorvos-ethyl	0.070	—	0.21	0.84	0.098
dimefox	0.52	0.70	0.95	0.77	0.60
EPN	0.070	0.24	0.28	0.63	0.11
malathion	0.98	1.6	1.1	1.4	12
paraoxon	0.035	0.12	0.19	0.49	0.07
parathion	0.052	0.16	0.49	1.8	0.11
parathion-methyl	0.042	—	0.22	0.39	0.14
phorate	0.16	—	0.28	0.95	0.38
"Potasan"	0.31	1.0	0.98	2.3	1.0
thiometon	0.28	—	0.56	0.70	1.2

Resistance spectra for the organo-phosphorus compounds have been drawn on the basis of the data in Table II and are shown in Figure 1, which calls for the following comments:

(1) Despite continuous selection for 100 generations, the "DFP" strain does not show high resistance levels nor does it show much specificity. This situation suggests that it has merely acquired vigour tolerance. On the other hand, its resistance levels remained fairly constant, in the absence of selection, for over a year; whereas the vigour tolerance of most laboratory selected strains is soon lost.

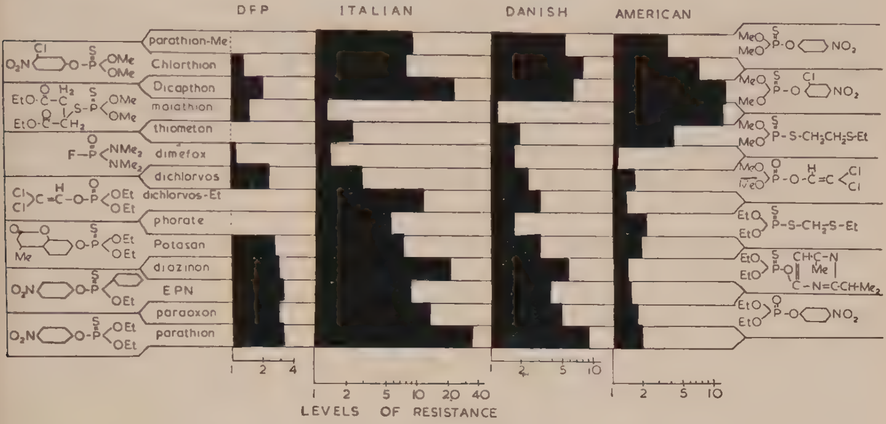

Figure 1. "Resistance Spectra" of various strains of housefly. The levels of resistance indicated are

$$\frac{LD_{50} \text{ resistant strain}}{LD_{50} \text{ normal strain}} \text{ for each compound.}$$

(2) The fly colonies originating in the field show considerably higher levels of resistance than the selected laboratory colony "DFP", probably as a result of selection from a larger genetic potential. If the resistance patterns of these three colonies are examined, it will be seen that the two European strains show definite similarities distinguishing them from the American strain. In particular, both of them show high resistance to parathion, paraoxon and diazinon and low resistance to malathion; whereas the American strain shows low resistance to parathion, paraoxon and diazinon and high resistance to malathion. It is not suggested that this reflects a basic difference in houseflies from Europe and America, but rather that the accident of insecticide usage has elicited a different type of resistance mechanism. There is, indeed, a little field evidence to suggest that the use of diazinon produces a resistance not involving malathion. For example, in the Belgian Congo, the blowfly *Chrysomyia putoria* Wiedemann could be controlled by malathion after developing resistance to diazinon, parathion and "Chlorthion". After some months, the flies developed resistance to malathion, but meanwhile lost some of their tolerance of diazinon and "Chlorthion" (BERVOETS, BRUAUX, LEBRUN & RUZETTE, 1957).

In the colonies I have investigated, diazinon selection (in Italy) evoked resistance to parathion and *vice versa* in the Danish strain. This is true of several colonies examined by OPPENOORTH (1958b); but he also found one colony ("A") selected by diazinon, which showed very low resistance to parathion and paraoxon.

An examination of the structural formulae of compounds involved in the various types of resistance (in Figure 1) does not provide a simple clue to the resistance mechanisms. The data confirm OPPENOORTH's (1958b) observation that resistance to organo-phosphorus compounds cannot be due to a failure to convert the inactive thiophosphates into phosphates, since the fly strains are more or less resistant to both types of compound. It appears that the American strain shows marked resistance only to compounds which combine a sulphur atom on the phosphorus in combination with two methoxy groups; but this may be fortuitous.

Another fact emerges from a close comparison of the Danish and Italian strains. The latter generally shows higher resistance; but the difference is greater in compounds with ethoxy groups attached to the phosphorus atom, rather than methoxy groups (with one marked exception) as shown in Table III. It was noticed some years ago that organo-phosphorus compounds containing $(CH_3CH_2O)_2P$— groups are generally more insecticidal than their homologues containing either methyl or propyl substitutes. METCALF (1948) suggested that steric considerations were involved, since the most potent compounds generally contained di-ethoxy, di-isopropyloxy or dimethylamino groups, all of which are about the same size. It is now generally agreed that steric factors limit the activity of the larger alkyl groups; but other factors are, perhaps, more critical for the ethyl and methyl groups (for example, the greater stability of the phosphorilated enzyme with an ethyl homologue: ALDRIDGE, 1953). The specific differences in toxicity between ethyl and methyl compounds are not large, however, and there are exceptions, such as the boll weevil (IVY, RAINWATER, SCALES & GORZYCKI, 1953). Therefore it has been suggested that their relative potencies reflect specific differences in enzyme properties. This may be relevant to the resistance characteristics of the Italian flies, which may be associated with changes in enzymes. VAN ASPEREN & OPPENOORTH (1959) have evidence on this point in the reduced ali-esterase content of all the resistant fly colonies.

TABLE III

Comparative resistance of the Italian and Danish strains to organo-phosphorus compounds, in relation to the alkyloxy groups. In all cases the compounds contain di-alkyloxy groups, except EPN and dimefox.

Ethoxy compounds		Methoxy compounds	
Compound	Ratio : LD Italian / LD Danish	Compound	Ratio : LD Italian / LD Danish
parathion	3.6	parathion-methyl	1.7
paraoxon	2.6	malathion	1.2
EPN	2.3	"Dicapthon"	3.1
diazinon	3.6	"Chlorthion"	1.0
"Potasan"	2.3	dimefox	0.8
phorate	3.4	thiometon	1.3
dichlorvos-ethyl	4.0	dichlorvos	1.2

Comparative Resistance to Injected Insecticide

A certain number of tests were conducted with the insecticide solutions injected into the flies (in the back of the thorax). Control mortality resulting from injection of the standard volume of olive oil (0.35 mm^3) was about 10%. The results were treated in the same way as those from application tests and they also gave straight probit/log. dose curves. Medial lethal doses for parathion and paraoxon were estimated for certain strains and are shown in Table IV.

TABLE IV

Relative resistance of various fly strains to parathion and paraoxon, by external application and by injection.

Treatment		LD$_{50}$ (μg/fly) for various strains				
		Normal	Italian		"DFP"	
Application	parathion	0.052	1.8	(× 35)	0.16	(× 3.1)
	paraoxon	0.35	0.49	(× 14)	—	
Injection	parathion	0.021	0.26	(× 12)	0.028	(× 1.3)
	paraoxon	0.0031	0.010	(× 3)	—	

It will be observed that the levels of resistance to injected poisons are all lower than corresponding figures for external treatment. This was observed some years ago by CHADWICK (1954) and myself (BUSVINE, 1957), working with the "DFP" strain. It was therefore tempting to suppose that this type of resistance was due to reduced penetration of the cuticle, especially when LORD & SOLLY (1956) found no difference in rate of detoxication of paraoxon between this strain and a normal one. However, it will be seen that the resistance to paraoxon is depressed much more than resistance to parathion, which agrees with OPPENOORTH'S (1958) observations with the parent colony ("D") of my Danish strain. I believe that he is right in rejecting the idea of a selective cuticular barrier and ascribing the loss of resistance to injected paraoxon to the excess of this rapidly acting poison swamping the protective mechanism. Indeed, it seems possible that this may occur to some extent with the normal colony, since the LD$_{50}$ of injected paraoxon is $\frac{1}{11.3}$ of the external dose, as compared with $\frac{1}{2.5}$ with parathion. It will be recalled that LORD & SOLLY (1956) found rapid detoxication of paraoxon by normal as well as resistant flies.

CONCLUSIONS

Unfortunately, the main conclusion to be drawn is that resistance to organo-phosphorus compounds is by no means a simple phenomenon. In three resistant strains, originating in the field, there was evidence of two distinct types of defence mechanism; and even the similar forms showed minor differences not attributable to chance.

Perhaps this is not surprising in view of the many processes involved in the mode of action of organo-phosphorus poisons, several of which may offer possibilities of altering the final result. Often these processes are conflicting, so that the ultimate toxicity depends on the balance struck. Often this is a compromise between high chemical activity, promoting rapid enzyme poisoning (but also inviting hydrolytic detoxication of the poison) and less active compounds which may form more stable, blocked, phosphorilated enzymes. For example, in the series $(MeO)_2$—P, $(EtO)_2$—P, $(Pro)_2$—P, the increasing degree of electron release of the alkyl group lowers the reactivity of the phosphorus atom, both to solvolysis and to reaction with esterase; but the stability of the phosphorilated enzyme greatly increases.

Since the resistance levels are not particularly high and since they are substantially reduced by the excessive poison present after injection, it seems probable that resistance is caused by a change in the rate of one or more steps in the poisoning process, perhaps associated with changes in enzyme specificity.

ZUSAMMENFASSUNG

Formen der Insektizid-Resistenz gegen organische Phosphorverbindungen bei Stubenfliegen-Stämmen verschiedener Herkunft

Vier gegen organische phosphorhaltige Insektizide resistente Stubenfliegen-Stämme wurden zusammen mit einer normalen Kolonie in Laboratoriumkultur gehalten. Die Resistenz des einen Stammes hatte sich durch Selektion im Laboratorium entwickelt, die der anderen durch den Feldgebrauch von Insektiziden und nachfolgender Selektion im Labor. Sie stammten aus Dänemark, Italien und USA, hauptsächlich nach Anwendung von Parathion, Diazinon bzw. Malathion. Die „Resistenzspektren" dieser Stämme wurden durch Messung ihrer relativen Resistenz gegenüber 14 organischen Phosphorverbindungen mit Hilfe eines Mikrotropfen-Applikator-Tests bestimmt. Die durch Parathion und Diazinon selektierten Stämme ergaben ziemlich ähnliche resistente Eigenschaften; sie zeigten höchste Resistenz gegen Parathion, Paraoxon und Diazinon und niedrige gegen Malathion. Der amerikanische Stamm ergab abweichendes Verhalten mit hoher Resistenz gegen Malathion und niedriger gegen Parathion, Paraoxon und Diazinon. Es wird vermutet, daß diese beiden Typen auf verschiedenen Resistenzmechanismen beruhen.

Ein eingehender Vergleich der zwei europäischen Stämme erbrachte größere Unterschiede in Verbindungen mit $(EtO)_2P$-Gruppen als in solchen mit $(MeO)_2P$-Gruppen.

Injektion von Parathion verminderte seinen Resistenzspiegel beträchtlich und die Resistenz gegen Paraoxon wurde bei Injektion noch mehr reduziert.

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FAT METABOLISM AND TEMPERATURE ACCLIMATIZATION IN THE FLY *PHORMIA TERRAENOVAE* R.-D.

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When larvae of the fly *Phormia terraenovae* were fed on diets containing fats with different melting points and degrees of saturation, the fat laid down in the depots were effected, though the range of the depot fats was much narrower than that of the fat in the diet. Larvae reared at high temperatures also laid down fat which had a higher melting point and a lower iodine number than did larvae reared at low temperatures.

No relation between the properties of the fat and the thermal death point was discovered, though the temperature of rearing had an effect.

Various insects are known to be able to become acclimatized to different temperatures, as a result of which the position of their thermal death points may be altered (Mellanby 1954). Those individuals of a species previously kept under warm conditions can survive exposure to higher temperatures than individuals previously kept under cool conditions. Similar reactions have been found in other poikilotherms (e.g. fish, see Fry, Hart & Walker 1946). The mechanism of this acclimatization has not been elucidated, but various workers have suggested that the composition of the body fat may be an important factor (Fraenkel & Hopf 1940, Heilbrunn 1952). Insects reared at high temperatures have been found to contain fat with a higher melting point and a lower iodine number than others of the same species reared at a low temperature (Rainey 1937).

It is well known that in many animals the type of fat in the diet determines the nature of the reserves laid down; there is usually some change in the fat, but generally the higher the melting point of the fat eaten the higher the melting point of that accumulated in the bodily depots. In some cases it has been found that animals which accumulate fat with a high melting point are relatively resistant to high temperatures compared with those with depots containing fat with a low melting point (Hoar & Dorchester 1949), but in other cases, as for instance the cockroach *Blattella germanica* (L.), no differences in thermal death point were found (Munson 1953).

This paper describes experiments in which blow-fly larvae were bred under various conditions and on different diets, so as to produce individuals with tissue fats of different degrees of saturation (and with different melting points). The relation between these changes in fat composition and the thermal death point of the larvae was then studied.

While this paper was being prepared for publication, an account of somewhat similar experiments on the larva of another fly, *Pseudosarcophaga affinis* (Fall.), appeared (HOUSE, RIORDAN & BARLOW 1958). The results of both series of experiments are discussed here.

METHODS OF BREEDING

(a) *Stock culture*: — The *Phormia* which were originally obtained from Silwood Park, the Field Station of the Imperial College of Science & Technology, were bred according to a technique used in the Department of Biology, John Hopkins University, Maryland, U.S.A., kindly communicated by Professor. V. G. Dethier.

Adult flies were kept in cages approximately 45 × 30 × 30 cms with two walls of glass and two of terylene net, in an outdoor insectary. This was heated during the winter months, when this work was done, but the temperature was not accurately controlled, and fluctuated over the course of each twenty-four hours, the highest and lowest readings noted being 29° C and 1° C; it seldom approached these limits, and the mean was around 18° C.

Food for the adult flies consisted of a mixture of equal parts by weight of granulated sugar and powdered half cream milk, spread in a petri dish and renewed daily. Drinking water was also provided.

Laying material was made up of a mixture of equal parts by weight of powdered half cream milk and dried brewer's yeast, mixed into a thick soup with hot water and set to a fairly stiff jelly with agar. While still fluid this was poured into 5 × 2½ cm specimen tubes which were corked and stored in the refrigerator. Each day the contents of one tube was sliced into rounds about 3 mm thick which were laid on moist filter paper in the bottom of a petri dish. This was placed in the cage and the lid of the petri dish tilted over it so as to cut down evaporation and desiccation.

For breeding from eggs the same milk-yeast mixture was prepared, but poured into an enamel basin 15 cm in diameter and allowed to set at room temperature. Small shallow holes were then scooped in the surface and freshly laid eggs placed in these and lightly covered with the scooped out material. The whole surface was then covered with one or two inches of sawdust and the basin placed in a biscuit tin with perforated zinc lid. When fully grown the larvae pupated in the sawdust and were either picked out and placed in a cage to emerge or the entire basin was transferred to a cage until emergence was complete. Larvae and adults did very well under these conditions provided they were not crowded; about 200 larvae was the optimum for each basin.

(b) *Experimental cultures*: — *Phormia* larvae were grown on herring, beef, mutton and pork by placing eggs from the stock culture on meat in basins stood in sand in biscuit tins with perforated zinc lids. The meat was replenished during growth, and when full grown the larvae crawled out into the sand and could be collected from it before pupation. In all experiments larvae which had left the food material and had empty guts but had not ceased crawling were used.

ANALYSIS OF LARVAE

(a) *Fat extraction*: — Previous workers have used many different methods for killing insect larvae, for drying them and for extracting the fat. A great deal of time was spent testing various methods (e.g. drying at 56° C, or at 103° C) and various solvents (e.g. ether, alcohol, acetone). The following method involving freeze drying was found to give the most consistent results, and was used throughout the investigations.

Phormia larvae were washed quickly in luke warm water with a little Teepol added, rinsed well in distilled water and dried on filter paper. They were then put into specimen tubes which were placed in a deep freeze unit at —23° C until frozen solid. The larvae were then quickly cut into small pieces about $^1/_{16}''$ thick with fine dissecting scissors and returned to the deep freeze for a few minutes before being placed in a cooled desiccator over phosphorus pentoxide. This was then evacuated to a pressure of approximately 1 mm Hg. The rate at which drying proceeded depended on the size of the pieces into which the larvae were cut, and was probably complete in a few hours when these were small, but the desiccator was generally left under vacuum overnight. This method gave similar percentages of water and dry matter to those obtained by drying in air at 103° C.

The dried larvae were placed in a Quickfit micro-Soxhlet apparatus with 15 mls. of light petroleum (B.P. 40°—60° C) added, and extracted on a steam bath for three hours (extraction for a further three hours yielded negligible amounts of fat). The Soxhlet apparatus was well rinsed with light petroleum into a weighing bottle of 35 ml. capacity, and the solvent evaporated off on the steam bath. The weighing bottle was dried in a desiccator and weighed to give the weight of fat obtained. The fat was redissolved in light petroleum and made to standard volume and stored in a refrigerator. Iodine numbers were done as soon as possible, generally within a day of fat extraction.

(b) *Determination of Iodine number*: — Several techniques were tried. The micromethod of Yasuda proved reasonably satisfactory, but it was difficult to ensure consistent results. On the suggestion of Dr. C. H. Lea of the Low Temperature Research Station, Cambridge, TRAPPE's (1938) modification of the method of Kauffmann was tried, and was found very satisfactory. With minor modifications it was used for all iodine numbers reported here.

A balance sensitive to ± 0.1 mg was used, so a minimum of 20 mgms. of fat for each determination was aimed at. This was not always available, but in the vast majority of cases between 20 and 30 mg was extracted. This was dissolved in 2 mls. chloroform and 2 or 3 mls. of brominating reagent (bromine dissolved in a saturated solution of sodium bromide in methyl alcohol) were added. After standing stoppered for 15 minutes, 2 mls. of 10% potassium iodide were added and the liberated iodine titrated with n/50 sodium thiosulphate with starch solution as indicator. As even freshly made up potassium iodide sometimes had a titer, a blank was run before each batch of determinations. The difference between blank titration and titration when fat was present gave a figure from which the iodine number was calculated.

DETERMINATION OF THE THERMAL DEATH POINT

A method similar to that described by MELLANBY (1932) was used. Larvae were placed, in batches of 5, in cylindrical cages made of stainless steel wire gauze, 1 cm in diameter and 4 cm long. The ends of the cylinders were closed with corks. These cylinders containing the larvae were placed inside glass boiling tubes submerged 20 cms below the surface of the water of a tank whose temperature was thermostatically controlled to within ± 0.1° C. The temperature of the cage was found to agree exactly (within ± 0.1° C) with that of the water temperature. The air during exposure was saturated (RH 100%).

Larvae were exposed to a particular temperature for 1 hour, and were then placed in sand and returned to the insectary. Their condition immediately after exposure was noted, and they were examined daily thereafter to determine their further survival, pupation and emergence.

RESULTS

(a) *Fat analysis*: — Table I gives the results of rearing *Phormia* larvae on different diets, and under two temperature regimes. It will be seen that on the "milk/yeast" diet the iodine number was 62. Larvae fed on pork, mutton and beef all had fat with an iodine number around 70. Larvae fed on herring had an iodine number of 90.

TABLE I

Analysis of larvae reared on different foods and at different temperatures

Expt. No.	Culture medium	Av. live wt. per larva mgms.	Av. dry wt. per larva, mgms.	Av. % water	Av. wt. fat per larva. mgms.	Fat % live wt.	Fat % dry wt.	Iodine number
				Bred in outdoor insectary (circa 18° C)				
189/×2	Milk yeast	88.0	24.3	72.4	6.1	6.9	25.1	62
189/×17	„ „	78.3	21.6	72.5	5.8	6.8	24.6	62
174/×4	Pork	80.5	22.8	71.7	6.8	8.4	29.7	70
174/×16	„	84.4	28.4	66.5	5.9	7.0	20.9	71
190/×10	„	—	—	—	7.1	—	—	68
190/×7	„	—	—	—	5.5	—	—	71
166	Mutton	79.0	—	—	6.7	8.4	30.9	71
175/×13	„	75.5	30.3	59.8	9.2	12.2	30.4	71
192/×15	„	—	—	—	6.6	—	—	71
192/×18	„	—	—	—	6.2	—	—	71
176/×2	Beef	87.8	25.7	70.7	6.9	7.9	26.9	70
176/×15	„	86.9	24.2	72.2	7.5	8.6	30.9	68
176/×17	„	82.9	24.1	70.9	7.2	8.7	30.0	69
176/×18	„	91.3	25.9	71.6	7.4	8.1	28.4	67
191/×3	Herring	76.0	20.7	75.7	5.7	7.5	27.5	90
191/×12	„	75.2	21.2	71.9	6.1	8.1	28.8	90
191/×19	„	75.8	21.7	71.3	6.1	8.1	28.1	90
				Bred at 35° C				
×8	Mutton	58.0	17.6	69.6	4.7	8.1	26.4	64
×10	Beef	79.3	23.3	71.5	7.0	8.8	30.0	65

No determinations of the iodine numbers of the fats in the food were made, but figures were obtained from the literature. It will be seen (Table II) that the diet had a considerable effect on the composition of the fat laid down by the larvae, but that some modification took place, so that the larval fat was more nearly uniform than that supplied in the diet.

TABLE II

The relation of the iodine numbers of food fat to that of tissue fat in Phormia *larvae*

Source of fat	Reference	Iodine number of food fat	Culture medium present experiment	Iodine number larval fat
Dried separated milk	Hilditch 1956	42.5	dried half cream milk and yeast	62
Butter and yeast	Yuill & Craig 1937	30		
Pig-composite body fat	Ellis & Isbell 1926	58.8		
Pig-outer back fat	Hilditch, Lea & Pedelty 1939	60.0	pork chop	70
Herring oil	Hoar & Dorchester 1949	128.4		
Whole herring October	Lovern 1938	129.9	whole herring	90

The larvae bred at the high temperature, 35° C, were slightly smaller than the others, but they contained a similar percentage of fat. This had a significantly lower iodine number than that found for larvae reared on the same food at a lower temperature.

(b) *Thermal death points*: — Previous work (K. MELLANBY, personal communication) had shown that *Phormia* larvae from various conditions and fed on several diets survived unharmed and produced normal adults after an exposure of one hour to 44° C. Experiments described here were therefore carried out at higher temperatures than 44° C. In preliminary experiments large numbers of larvae were used, but for the final tests rather smaller numbers, reared under controlled conditions were available. Thus for the experiments at 47° C, 195 larvae were used to establish the data given below.

After 1 hour at 45° C all larvae reared in the outdoor insectary (circa 18° C) on milk yeast, beef, pork, mutton and herring appeared normal in their behaviour and activity. All pupated, and the majority gave normal adults.

Similar larvae were exposed to 46° C for 1 hour; some individuals when removed from the water bath were sluggish, but they soon recovered and all gave pupae. Only 81% of the larvae gave normal adults, however.

After 1 hour at 47° C the larvae bred at around 18° C on all the diets did not appear normal. They were either abnormally over active, and uncoordinated in their movements, or they were quite inert. The majority appeared to recover, however, and 79% pupated. Only 34% gave rise to normal adults.

After 1 hour at 47.5 C all larvae bred around 18° C were quite inert. Most individuals never recovered, but 23% formed apparently normal pupae. Only one single adult fly emerged from all those exposed to this temperature.

In the above experiments, larvae fed on milk yeast, beef, pork, mutton and herring were used. The diet, and the iodine number of the fat, seemed to make little or no discernible difference to the thermal death point.

Phormia larvae bred at 35° C were also exposed to 47° C and 47.5° C. After 1 hour at 47° C 64% gave normal adults. After exposure to 47.5° C most of these larvae were still active at the end of 1 hour, though they were not quite normal in their behaviour. 55% pupated and 33% gave normal adults. These larvae bred at 35° C were clearly more resistant to heat than those bred at 18° C. (See Table III). It did not appear to make any difference whether the larvae bred at 35° C had fed on beef or mutton.

TABLE III

Results of exposure to high temperatures of Phormia *larvae bred at different temperatures*

Exposure for 1 hour to	Larvae bred at 18° C			Larvae bred at 35° C		
	Behaviour	% Pupating	% becoming Adult	Behaviour	% Pupating	% becoming Adult
46° C	Normal	100	81	—	—	—
47° C	Abnormally active or inert	79	34	Normal	84	64
47.5° C	Inert	23	0	Abnormally active	55	33

Discussion

These experiments show clearly that the nature of the fat in the food influences the nature of the fat laid down in the body of larvae of the fly *Phormia terraenovae.* These results are in accordance with results of previous workers (e.g. Lovern (1938) with the eel, Hoar & Dorchester (1949) with the goldfish, Melampy & Maynard (1937) with the cockroach). However, the variation in the degree of unsaturation of the fats laid down in the body (Iodine number of 62 for those fed on milk yeast, Iodine number of 90 for those fed on herrings) was much less than that found in the food ingested (Iodine number of 30 approximately for milk yeast, Iodine number of 130 for herring). This confirms the statement of Lovern (1938) "that there is in the animal a mechanism which can deal with ingested fat so as to modify it to the normal requirements of its depot fat".

The temperature of the environment in which an insect lives may affect the composition of the depot fat (e.g. Rainey 1937). This was found to occur with *Phormia.* Larvae reared at 35° C on mutton had an Iodine number 7 points lower than individuals fed on the same diet at 18° C. A similar difference was obtained from individuals fed on beef.

It has been suggested that the nature of the fat may play an important part in the mechanism of resistence of animals to higher temperatures. Recently HOUSE, RIORDAN & BARLOW have shown that larvae of *Pseudosarcophaga affinis* reared on a diet rich in unsaturated fats are less resistant to higher temperatures than those reared on a diet rich in saturated fats. This would appear to contradict the results reported here. It should be noted, however, that the difference in resistance HOUSE, RIORDAN & BARLOW found was very small. They express their results rather differently from the method used in the experiments described here. For a lethal temperature, they determine the time required to give a mortality of 50 per cent. Thus "unsaturated" *Pseudosarcophaga* larvae exposed to 45° C, gave a 50 per cent mortality in 177 minutes (5 per cent. limits 159 and 196) while 50 per cent. of "saturated" larvae survived for 218 minutes (5 per cent. limits 198—240 minutes). This is an increase in survival of approximately 20 per cent. This method of expressing resistance is a better one than that used here, but it needs far more material and is very time consuming. It has been shown (MELLANBY 1959) that the simpler method used here of the "one hour thermal death point" gives very nearly as much information with much less trouble. It is not impossible that the "unsaturated" *Phormia* are less resistant to high temperatures than the "saturated" individuals, but the difference, due to saturation, must be very small indeed. It should in addition be noted that the differences in fats found in my experiments (Iodine number ranging from 62 to 90) are much greater than those obtained by HOUSE, RIORDAN & BARLOW (64.3 to 75.5). If "saturation" of fat had an important effect on resistance to heat, this would surely have been obvious even using the comparatively crude "one hour thermal death point" technique.

On the other hand acclimatization has a substantial effect on thermal resistance. HOUSE, RIORDAN & BARLOW showed that "preconditioning" for only 2 hours to 39° C could increase the period required to give 50 per cent. mortality by nearly 50 per cent as compared with larvae maintained at 23° C. It seems unlikely that the composition of the body fat was altered during this brief exposure. I found a substantial increase in resistance in larvae reared at 35° C compared with those reared at 18° C, but this change was not in any way related to a proportional change in the nature of the fat in the larvae. The work of MELLANBY (1954) also suggests that rapid acclimatization alters heat resistance more rapidly than the fat depots could be suitably altered. Incidentally *Phormia* larvae, like *Pseudosarcophaga*, can be rapidly acclimatized, without the necessity for rearing right through the larval period as in these experiments (K. MELLANBY, personal communication).

These experiments confirm that diet can alter depot fat, and that environmental temperature can do so also, but they show that fat is not likely to be of major importance in controlling heat resistance in insects, if in fact it is of any importance at all. A difference in saturation of fat in insects with different thermal resistance may be a coincidence with no bearing on the mechanism of resistance at all.

ZUSAMMENFASSUNG

Fettstoffwechsel und Temperaturanpassung bei der Fliege Phormia terraenovae *R.-D.*

Larven der Fliege *Phormia terraenovae* R.-D. wurden im Insektarium bei annähernd 18° C gezogen und mit folgenden Nährstoffen gefüttert: Hefe/Milch, Schweine-, Hammel-, Rindfleisch, Hering.

Wenn die Larven völlig erwachsen waren, wurden sie getötet und analysiert. Die Larven wiesen nach allen Ernährungsformen ähnliche Zusammensetzung auf, mit Ausnahme der Jodzahl des Fettkörpers. Diese variierte folgendermaßen: Milch-Hefe-Diät = 62, Schwein = 70, Hammel = 71, Rind = 69, Hering = 90. Die Unterschiede zwischen mit Schwein, Hammel und Rind ernährten Larven waren nicht signifikant, die anderen Differenzen jedoch stark signifikant.

Die Unterschiede zwischen den Jodzahlen der Fette in den verschiedenen Nährstoffen waren größer als diejenigen, die in den mit ihnen gefütterten Larven gefunden wurden (Milch-Hefe = 30, Schwein = 60, Hering = 130).

Mit Hammelfleisch bei 35° C ernährte Larven enthielten Fett mit einer Jodzahl von 64 (gegenüber 71 bei den unter 18° C gehaltenen).

Der thermale Todespunkt war für alle bei 18° C gezüchteten Larven unabhängig von ihrer Ernährung ungefähr der gleiche. Die bei 35° C gehaltenen Larven wiesen einen annähernd um einen Grad höheren Todespunkt auf.

Es scheint also wenig oder gar keine Beziehung zwischen der Zusammensetzung des Fettkörpers der *Phormia*-Larven und ihrer Resistenz gegen höhere Temperaturen zu bestehen.

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THE FEEDING AND EXCRETION RATES OF *APHIS FABAE* SCOP. ON *VICIA FABA* L.

BY

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The feeding and excretion rates of nymphs of *Aphis fabae*, feeding on young leaves of *Vicia faba*, were studied, using host plants grown in water culture and made radioactive with 32P. The amounts of sap ingested at first were small but the rate of ingestion increased rapidly between 12 and 16 hr. The maximum rate of feeding was estimated at 0.2 mg sap/hr, an uptake of 59 per cent of the mean body weight of the insects per hour. The results are discussed in relation to other recent work on aphid feeding and excretion.

Studies of the amount of plant sap ingested by aphids and of their excretion of honeydew have been remarkably few. HAMILTON (1935) determined the gain in weight of groups of *Myzus persicae* (Sulz.) feeding on *Hyoscyamus niger* leaves and PATHAK & PAINTER (1958) similarly estimated the loss or gain in weight of *Rhopalosiphum maidis* (Fitch) when feeding on sorghum varieties; but in both cases no corrections were applied for losses due to evaporation and excretion. DAY & IRZYKIEWICZ (1953) and WATSON & NIXON (1953) estimated the uptake of sap by *M. persicae* by allowing the insects to feed on leaves made radioactive with 32P and then relating the radioactivity of the aphids to that of the leaves. MITTLER (1958a) estimated the rate of sap ingestion by *Tuberolachnus salignus* (Gmelin) from the willow (*Salix* spp.) on which it feeds by comparing the rate of flow of sap from the aphids' cut stylets embedded in the phloem with the rate of excretion of intact aphids.

DAY & IRZYKIEWICZ found only small amounts of radioactivity in the honeydew of *M. persicae* and *Brevicoryne brassicae* (L.) and WATSON & NIXON recorded only traces of radioactivity in the excreta of *M. persicae*. BANKS & NIXON (1958), however, found high levels of radioactivity in the honeydew of *Aphis fabae* Scop. nymphs feeding on bean leaves.

In the present experiments, separate measurements were made of the estimated uptake of sap and of the excreted honeydew of nymphs of *A. fabae* on radioactive bean plants, thus supplementing the earlier work. The method suffers from the defect that the radioactivity of the sap which the aphid actually ingests is unknown, but is assumed to be equal to that of a sample of the leaf tissue.

METHODS

Experiments were made in August and September 1957 and 1958 in an open air insectary in which environmental conditions were not controlled. The plants were subject to daylight but not to direct sunlight. Because adults lose radioactivity when they give birth to nymphs, a fact noted by WATSON & NIXON but apparently overlooked by DAY & IRZYKIEWICZ, nymphs of *A. fabae* were used in all our experiments. They were born from alate virginoparae on young bean leaves, and most were transferred to experimental plants as 3rd instar nymphs, a few being in the 2nd or 4th instar.

Bean seeds were germinated in wet sand or soil and transferred when the plants ware 4-6 in. high to individual bottles of Hoagland's water culture solution deficient in phosphorus. Before being used for feeding experiments, selected groups of plants received fresh culture solution to which 300 $\mu c/l$ of 32P had been added in the form of H_3PO_4 with a little NaH_2 PO_4 as a carrier. They remained in this solution until rough measurements with a G.M. tube attached to a rate-of-count meter showed that they were sufficiently radioactive. The plants were then transferred to fresh non-radioactive solution, and leaves of a similar age on all the plants in a group were selected for experiment. The leaf opposite to each one selected was then removed, and the prepared plants allowed to stand for 24 hr before use.

Aphids were removed from their host plants and groups of 20 were transferred to the bottom of fine mesh organdie bags, shaped to fit the selected leaves. The mouths of the bags were closed around the petioles with clips, any gaps being plugged with cotton wool. This operation took about 15-20 min. After a further 30 minutes, the bags were opened carefully so that any aphids which had not settled could be removed. The majority settled readily within a few minutes, particularly when the upper surfaces of the bags had been illuminated and the lower surfaces darkened with black paper. All feedings were timed from the introduction of the aphids into the bags.

After the aphids had fed for the desired time, the leaf in its bag was cut off the plant, turned upside down and pinned to a sheet of cork, care being taken to avoid contamination of the bag with sap from the cut end of the petiole. The bag was slit open and the aphids with any cast skins removed with a brush into a few drops of alcohol in a test tube. The bag with the excreted honeydew on it was put into another tube. A sample of leaf tissue, cut with a cork borer 12 mm diameter, was then taken from where the aphids had fed, weighed and put into a third tube. After gentle heating of the aphid samples on a water bath to evaporate the alcohol, a few drops of HNO_3 were added to each tube, which was then heated cautiously to dissolve the contents. After suitable dilution the extracts were assayed in a jacketed G.M. tube attached to a scaling unit. In each of the 12 experiments 6 groups of aphids were used, each group being fed on a different plant.

RESULTS

For any one feeding period, the estimated weight of sap ingested was calculated from the observed radioactivities of the aphids and that of their honeydew, which were then related to the specific radioactivities of the leaf samples. Mean estimates of the weight of sap ingested during each of the twelve feeding periods, which lasted from 1 hr to 24 hr (Table I), are plotted in Fig. 1.

The curve shows a low rate of ingestion from 1 hour to about 12 hours of feeding, but between 12 and 16 hours the rate increased very rapidly to about 0.2 mg sap/hr. The average weight of the aphids was 0.32 ± 0.03 mg, so that at the full rate of feeding the aphids were ingesting 59 per cent of their own

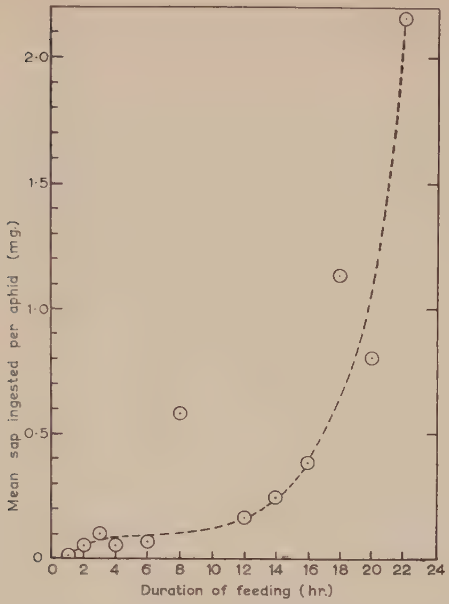

Fig. 1. Estimated mean weights (mg) of sap ingested by nymphs of *Aphis fabae* Scop., feeding for various periods on young, radioactive leaves of *Vicia faba* L.

weight per hour. The results of the 8-hour feeding period show a very low proportion of excreted 32P and a high radioactivity of the aphids in each of

TABLE I

Estimates of the amounts of sap ingested and of the proportion of 32P excreted by nymphs of Aphis fabae *feeding for various periods on young, radioactive leaves of* Vicia faba

Duration of feeding	Total aphids in 6 experiments	Estimated mean weight of sap ingested per aphid (± S.E.)	Proportion of ingested 32P which was excreted (± S.E.)
hr		mg	%
1	112	0.011 ± 0.002	62.8 ± 8.4
2	111	0.054 ± 0.010	16.6 ± 3.5
3	110	0.096 ± 0.007	11.9 ± 3.1
4	114	0.054 ± 0.016	44.2 ± 4.0
6	114	0.069 ± 0.016	56.0 ± 4.1
8	120	0.581 ± 0.146	3.8 ± 1.2
12	113	0.161 ± 0.038	31.4 ± 2.1
14	116	0.246 ± 0.038	35.1 ± 2.9
16	115	0.382 ± 0.050	40.9 ± 1.6
18	118	1.137 ± 0.010	22.4 ± 3.6
20	109	0.806 ± 0.298	45.0 ± 3.9
24	115	2.153 ± 0.464	42.9 ± 2.0

6 groups of nymphs. We are unable satisfactorily to explain these anomalous results.

The rate of excretion of the aphids varied considerably, but it was higher for the shorter feeding periods of less than 12 hours, and lower and less variable for the longer times.

DISCUSSION

The maximum rate of feeding of *M. persicae* recorded by WATSON & NIXON was 0.042 mg sap/hr, which was an uptake of only 10 per cent of the insects' body weight per hour (assuming an average weight of 0.4-0.5 mg). Our results for *A. fabae* are more like those obtained for *T. salignus* by MITTLER (1958b). For third instar nymphs, he recorded an uptake of sap equal to 54 per cent of the aphids' mean body weight, if we assume the rate of excretion to be equal to the rate of ingestion and take the density of the sap to be 1.0.

We have no information on the rate at which the aphids reached the phloem in our experiments. The experiments of ROBERTS (1940) with various aphid species showed that after a few hours of feeding, comparatively few aphids had penetrated to the phloem and that even after 24 hours, about one-quarter of *M. persicae,* for example, were still not feeding there. WATSON & NIXON observed a change in the feeding rate of *M. persicae* feeding on tobacco leaf, and suggested that this happened when the aphids reached the phloem. SKOTLAND & HAGEDORN (1958) also showed that the proportion of *Macrosiphum pisi* (Kalt.) (*Acyrthosiphon pisum* (Harris)) which reached the phloem increased with longer feeding times.

The feeding rate may have changed between 12 and 16 hours in our experiments because a gradually increasing proportion of the aphids reached phloem tissue where they may feed faster than on other tissue. Variations in the proportion of 32P excreted might merely correspond to changes in the concentration of the isotope in different kinds of plant tissue. But 12 to 16 hours seems a long time for the aphids to take to penetrate to the phloem where, it is generally thought, they normally feed. Previous workers have used adult aphids in their experiments, whereas we have used nymphs whose feeding behaviour may differ considerably from that of adults; nymphs may, for example, feed longer on other plant tissues before finding the phloem. On the other hand, adult aphids appear to reduce their rate of excretion (and, presumably, feeding) when giving birth to young, as AUCLAIR (1958) has shown for *A. pisum.* AUCLAIR also showed that the rate of excretion increased as nymphs of *A. pisum* grew. The aphids in our experiments were growing, and, therefore, an increasing proportion of them would have been feeding at a higher rate.

It is also possible, but in our view unlikely, that most *A. fabae* reached the phloem within a few hours and that the lower rate of ingestion recorded for the first 12 hours of feeding was in fact the "normal" one for our experimental conditions. The rate at 12-16 hours might then have changed from some other cause, such as a rise in turgor pressure within the plant, although we have no reason to suppose that this happened. We recognise that the turgor pressure must set a limit to the maximum possible feeding rate, but we reject the idea that the feeding rate is normally determined by the turgor pressure alone, for, as we have shown previously (BANKS & NIXON, 1958), *A. fabae* is able to increase its feeding and excretion rates when attended by the ant, *Lasius niger* (L.). It is also reasonable to suppose that the aphids could increase their feeding rates for some reason, perhaps in response to a deficiency of some substance in the sap (WATERHOUSE, 1957). For the time being, however, we incline to the view that the maximum rate of feeding recorded above was the "normal" under the conditions of the experiments, and that most of the nymphs took a long time to reach the phloem.

We are grateful to Mr. F. G. Smith and Mr. R. D. Woods for their help with the experiments.

ZUSAMMENFASSUNG

DIE NAHRUNGSAUFNAHME- UND EXKRETIONSRATEN VON APHIS FABAE *SCOP. AN* VICIA FABA *L.*

Unter Benutzung von Wirtspflanzen, die in Wasserkultur angezogen und mit 32P radioaktiv gemacht waren, wurden Nahrungsaufnahme- und Exkretionsraten der Larven von *Aphis fabae* untersucht, die an jungen Blättern von *Vicia faba* saugten. Die aufgenommenen Saftmengen wurden für Zeitabschnitte von 1—24 Stunden Dauer geprüft. Die maximale Nahrungsaufnahmerate wurde mit 0,2 mg/Stunden geschätzt. Die Exkretionsrate von 32P variierte beträchtlich, war aber bei kürzerer Nahrungsaufnahme von weniger als 12 Stunden Dauer höher, und bei längeren Nahrungsaufnahmezeiten niedriger und weniger variabel. Die Ergebnisse werden im Hinblick auf andere neuere Arbeiten über die Ernährung und Exkretion von Blattläusen diskutiert. Es wird vermutet, daß die maximale Rate der Nahrungsaufname, die für diese Insekten angegeben wird, unter den Bedingungen des Versuchs die „normale'' war und daß die Mehrzahl der Aphiden offenbar 12 Stunden oder mehr brauchen, um mit ihren Stechborsten bis zum Phloem vorzudringen.

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EFFECT OF PARASITIZATION BY *APHIDIUS PLATENSIS* BRÈTHES ON THE DEVELOPMENTAL PHYSIOLOGY OF ITS HOST, *APHIS CRACCIVORA* KOCH

BY

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Parasitization of nymphs of *Aphis craccivora* by *Aphidius platensis* results in interference with their normal developmental physiology. Different stages of growth of the parasite have opposite influences on the host: unhatched parasite eggs exert a "juvenilizing" effect, producing metathetely; whereas parasite larvae sometimes cause the premature appearance of adult characters (prothetely). When winged nymphs of the host are parasitized, the differentiation of alatiform structures is inhibited. This results in the production of aphids intermediate in form between normal alatae and apterae; the extent of development of alatiform structures is determined by their degree of differentiation at the time of parasitization and whether one or more parasite eggs are deposited. It is suggested that parasitic metathetely in aphids is brought about by interference with the titre of juvenile hormone, and that alate-apterous polymorphism in aphids generally is controlled by the same hormone system as is concerned in moulting and metamorphosis.

When the immature stages of some insects and crustacea are parasitized, the ensuing adults have been found to develop certain structural abnormalities. "Parasitic castration" of some crabs is caused by isopod and rhizopod infections: male crabs tend structurally towards the female type and female crabs retain some juvenile characters (CAULLERY, 1952). In insects, parasitization of various Hymenoptera and Homoptera by Strepsiptera causes the production of "intersexes", the particular sex that is parasitized developing some of the characteristics of the opposite sex (CLAUSEN, 1940). *Mermis* infections of larvae of worker ants result in the development of ocelli and other characteristic queen structures (WHEELER, 1937). *Mermis* can also cause restriction in wing size in the alate sexual forms of ants (MRAZEK, 1908). Structural deformities of alate ants, including complete suppression of the wings, are brought about by parasitization by the chalcid wasp *Orasema* (WHEELER, 1910). In simuliid larvae STRICKLAND (1911), showed that parasitization by *Mermis* resulted in suppression of the development of the imaginal discs. This phenomenon of the suppression of adult structures only, he termed metathetely. Instances of metathetely have been found by HALCROW (1954) in *Anopheles* larvae parasitized with *Glaucoma pyriformis* (Ehrenberg) and by FINLAYSON & WALTERS (1957) in saturniid moths infected with *Nosema.* It has been suggested for cases of parasite-induced metathetely that the hormone system of the host has been in some way upset by the parasites (WIGGLESWORTH, 1954; FINLAYSON & WALTERS, 1957).

In the present paper an account is given of the influence of parasitization by the braconid wasp *Aphidius platensis* Brèthes on the morphology of its host *Aphis* *craccivora* Koch. A brief account of the work has already been published (JOHNSON, 1958). The bionomics of wasp parasites of aphids have been studied by a number of workers but there does not appear to be any mention in the literature of an influence of the parasite on the morphology of the host, apart from a brief remark by WEBSTER & PHILLIPS (1912) that the wings of aphids parasitized after the second moult are often imperfect. VEVAI (1942) noted that the "sessiles" or "mummies" of dead parasitized aphids are nearly always apterous, but possibly because he had already found that wasps oviposit more readily in apterous than in alate adult aphids he did not look for the cause.

MATERIALS AND METHODS

The aphids used in this study were alienicolae of *Aphis craccivora* Koch which were reared on broadbean, *Vicia faba* L. Batches of aphids of uniform known age were obtained by keeping a number of adults on bean cuttings overnight and removing them the next morning and rearing the progeny which had been deposited on the cutting. Alatiform nymphs were obtained by putting apterous parents on cuttings, bearing only one mature leaf from old bean plants. The cuttings were kept in jars of water. When reared under these conditions the progeny of apterae nearly all developed into alates. The results of five such rearings are given in Table I.

TABLE I

Form of progeny of apterous parents when reared on mature bean leaf cuttings in water

Experiment No.	Total No. of larvae	% alatae
1	391	98
2	296	97
3	126	83
4	174	88
5	391	92

Apteriform nymphs were obtained as the progeny of young alate parents born within 24 hours after the parents had settled on a host plant: these progeny invariably developed into apterae.

The parasite used was *Aphidius platensis* Brèthes, adults of which were kindly identified by Dr. C. F. W. Muesebeck of the National Museum, Washington, D.C., U.S.A.

Aphids to be parasitized were put on a leaf and kept in a small glass cage containing a number of wasps. When parasitization with a single wasp egg was required, the aphids were placed individually on a leaf and removed as soon as a wasp had thrust at one of them with its ovipositor: dissections of a number of aphids treated in this way failed to reveal the presence of more than one egg. Aphids left in the cage were repeatedly oviposited in by the wasps: the maximum number of eggs found in an aphid which had been left in the cage overnight was fifty-seven. This is in accord with the observations of a number of authors who have found that braconid wasps will oviposit in aphids in which they or other wasps have already deposited eggs.

The parasitized aphids were reared on bean cuttings in water. This provided an adequate nutrition source for the aphids to attain the same speed of development and size as aphids reared on seedlings.

The morphological structures of the aphids were examined in the intact aphids immersed in 70 % alcohol, and where a more detailed study of their cuticular structures was required the aphids were later cleared in chloral phenol and mounted in gum arabic on microscope slides.

LIFE CYCLE OF *APHIDIUS PLATENSIS* BRETHES

The life cycles of several species of *Aphidius* have been studied by a number of authors (SPENCER, 1926; ULLYETT, 1938; VEVAI, 1942, etc.). The biology of *A. platensis* does not appear to differ in any significant way from the accounts of other species which have been published.

An egg tapering at both ends and measuring about 20 × 50μ is deposited in the body cavity of the aphid by the female wasp. Development of the egg of *A. platensis* resembles closely the description of the development of eggs of Aphidiinae given by SPENCER (1926). The parasite eggs begin to swell soon after being laid, and ten hours after oviposition they contain many nuclei. At twenty-four hours at 20° C the eggs have increased to about 80μ in diameter and the embryo resembles the "morula" stage of vertebrate embryos. At forty-eight hours the egg measures about 160-200μ in diameter and contains an undifferentiated elongated worm-like embryo curled about on itself but which when straightened out measures about 500μ in length. During the third day the size of the head increases, external segmentation of the body occurs and tissue differentiation is initiated: for the first time the nervous system, alimentary canal and salivary glands can be recognized as distinct structures. At the time of hatching the egg measures about 250μ in diameter. After hatching, the cells of the serosa become greatly enlarged and continue their existence as free-living cells in the aphid until they are finally consumed by the parasite. The parasite larva has well developed mandibles but apparently obtains most of its food from imbibing the host's haemolymph, as it is rarely found with its mandibles imbedded in host tissues and the host tissues rarely show evidence of physical injury. The first and second larval instars, which can be identified by the length of the caudal process, last for only about one day each at 20° C. Then follow about three further instars (VEVAI, 1941) in which the appearance of the parasite does not change appreciably from one instar to the next except that it becomes more curved about on itself; these last for a total of 3-4 days. Soon after the last larval stage is reached, the larva changes its feeding habits and voraciously cleans out the entire body contents of the host; this is facilitated by the secretion of cytolytic enzymes which cause breakdown of the host tissues. The parasite then spins a cocoon inside the aphid skin and pupates.

When more than one egg is deposited in an aphid, all hatch in about the same time as a single egg, but all but one of the resulting parasite larvae die within a few days. The larvae that die appear to be killed by chemical means rather than by physical injury caused by the surviving parasites since their cuticle frequently remains intact well after the body contents have completely lost their structure.

NORMAL DEVELOPMENT OF THE HOST

There are four nymphal instars in both alatiform and apteriform nymphs of *Aphis craccivora* Koch. When reared on healthy bean plants at 20° C the approximate duration of the successive instars in apteriform nymphs is 1.5, 1.5, 2.0, and 2.0 days; in alatiform nymphs, the last stadium is typically one day longer.

Externally, nymphs which will develop into alatae do not differ from those which will develop into apterae until the third nymphal cuticle is laid down. In third instar alatiform nymphs, small wingpads are present and the thorax is distinctly alatiform in character: the mesothorax is lengthened and broadened and the metathorax shortened, giving the thorax a more box-like appearance than in apteriform nymphs, where the segments remain approximately the same length and taper in width towards the head. In the fourth instar the difference between the two forms is more pronounced: apteriform nymphs are merely larger but not different in body shape from the previous instar, whereas in alatiform nymphs, the wingpads are much larger and the thorax is more conspicuously different in its shape and dimensions from that of apteriform nymphs.

Adult aphids differ from nymphs principally in that they are reproductively mature. There are a number of structural features which distinguish nymphs from adults: the length and proportions of the appendages, the shape of the cauda and the presence in the adult only of a genital aperture and genital plate. Areas of pigmentation also differ on the two forms. The whole of the dorsal surface of the head capsule is pigmented in adults, whereas in nymphs, the pigment is confined to two broad bands running longitudinally along it; in adults the dorsal surface of the abdomen is pigmented, whereas in nymphs, it is unpigmented.

Adult alate aphids differ structurally from apterous adults in a number of features, among which are the presence in alatae and absence in apterae of wings, specialized pterothoracic sclerites, ocelli, and placoid sensoria on the third segments of the antennae. The antennae also differ in the two forms in the relative lengths of the 2nd and 3rd segments, and in their degree of pigmentation: in alatae, the antennae are pigmented along their whole length, whereas in apterae, they are pigmented at the ends only, the intermediate region being unpigmented. The pigmentation of the abdomen of the two forms also differs: in apterae, there is a dorsal pigmented "shield" covering the entire abdomen and thorax, whereas in alatae there are discrete bands of pigment across of the abdominal sclerites.

RELATIVE RATES OF DEVELOPMENT OF HOST AND PARASITE AT 20° C

At 20° C the parasite egg hatches on the third day after oviposition. Thus eggs deposited in aphid nymphs do not hatch until the nymphs have undergone considerable growth and have moulted once or twice since oviposition. In Fig. 1 a schematic representation is given of the relative rates of development of host and parasite at 20° C when the aphid is kept on a host plant that favours optimum development. On less favourable host plants, the speed of development of the aphids is retarded but parasite development is not affected.

No detailed study has been made of the effects of different temperatures on the relative rates of development of host and parasite, but incidental observations suggest that they are not very extensive at least at temperatures within the range of 15-25° C.

Webster & Phillips (1908) and Ullyett (1938) found that when aphid nymphs were parasitized before the first or second moult, both aphids and parasites failed to survive. In the present study, adult wasps emerged from all aphids that were parasitized, regardless of the instar of the host at the time of parasitization, provided the aphids received adequate nutrition. All nymphs that were parasitized in the first instar failed to moult into adults and were mostly killed by the parasite in the fourth instar. The number of moults aphids underwent after oviposition and before they were killed by the parasite larvae was determined by the nutritional status of the host plants they were reared on. Inadequate nutrition for aphid development, within limits, did not interfere with parasite growth. When parasitized nymphs were kept on inferior host plants, the parasites frequently completed their development and killed the aphids in the third and fourth instars even when oviposition occurred as late as the second and third instars respectively; on suitable host plants aphids parasitized in these instars developed to maturity, before they were killed.

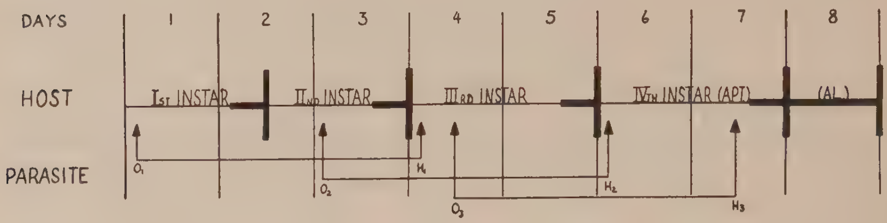


Fig. 1. Relative rates of development of host and parasite at 20° C. The heavy horizontal lines indicate the approximate duration of the moult from the beginning of endocrine activity. O: oviposition; H: hatching of the parasite egg.

PARASITIZATION OF APTERIFORM NYMPHS

Apteriform nymphs parasitized at all stages continued their development normally without undergoing any major structural changes. Those which were parasitized in the first instar were killed and "mummified" by the parasite in the fourth instar and a number of them showed some premature development of adult characters (prothetely), having the cauda intermediate in shape between nymphal and adult types, and patches of pigment on the abdomen at the lateral muscle insertions.

Nymphs parasitized in the second and subsequent instars developed to maturity, and the only apparent external effects of parasitization in the resulting adults was suppression of pigment deposition on the dorsal surface of the abdomen and the retention of slightly juvenile pigmentation pattern of the head capsule.

PARASITIZATION OF FIRST INSTAR ALATIFORM NYMPHS

The influence of parasitization on first instar alatiform nymphs was demonstrated in the following experiment. Five bean leaves bearing batches of first instar alatiform nymphs which had been born to apterous parents in the preceding overnight period were put in the parasite cage for six hours. The leaves were then removed from the parasite cage and the aphids were reared in another cage at an average temperature of 15° C with a control series of unparasitized nymphs. All the parasitized aphids were killed by the parasites when they were in the fourth nymphal instar. At the time they died they had no alatiform structures, although the control series consisted almost entirely of normal alatiform nymphs (Table II). The parasitized nymphs were indistinguishable from normal unparasitized apteriform nymphs except that some of them showed some degree of prothetely (Table III). Thus in many of them, the lateral abdominal muscle attachments were pigmented, and in a few there was a rudimentary, cleft genital plate, a cauda intermediate in size and shape between nymph and adult (fig. 2), and some pigmentation of the dorsal surface of the abdomen (fig. 17).

TABLE II

Effect of parasitization on the form of aphids parasitized in the 1st instar

	Alatiform N. IV	Apteriform N. IV
Unparasitized controls	254	27
Parasitized aphids	0	283

TABLE III

Numbers of aphids parasitized in the 1st instar showing different degrees of prothetely in the 4th instar

	Number of aphids
Total number of aphids	363
Normal apteriform nymphs	147
Nymphs with pigmented muscle insertions	216
Nymphs with intermediate shaped cauda	58
Nymphs with pigmented areas on tergites of abdomen	25

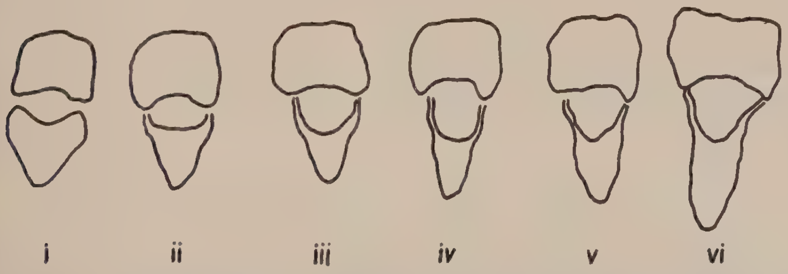


Fig. 2. Cauda and anal plate of i) normal fourth instar nymph; ii—v) parasitized fourth instar nymphs which had become parasitized in the first instar; vi) normal adult.

Several parasitized nymphs were dissected just prior to casting their third instar cuticle and they were found to contain second instar parasite larvae that had apparently only recently moulted from the first instar as they were still enveloped by the first instar cuticle.

PARASITIZATION OF SECOND INSTAR ALATIFORM NYMPHS

A number of alatiform nymphs which had moulted into the second instar overnight were placed the following morning in the parasite cage and left there for six hours. They were then kept in another cage with a control series of unparasitized aphids and reared to maturity. The aphids were collected and examined as they moulted to adults. A few of them were killed by the parasites while still in the fourth instar. All the parasitized aphids that survived to become adults developed characters intermediate between alate and apterous, but in general appearance they were closer to the apterous form. All of them had rudimentary ocelli, and the antennal sensoria were either lacking, or, when present were frequently very reduced in size and number. The degree of wing development varied: in some aphids there was no trace of pterothorax differentiation, in others the shoulders of the mesothorax were squared and small rudimentary wing evaginations were present. The head capsule was of the fully pigmented adult type and most of the aphids had some pigmentation of the dorsal surface of the abdomen (fig. 18; Table IV).

TABLE IV

Characters of intermediates formed from parasitization of 2nd instar alatiform nymphs

Characters	Number of aphids
Total number of aphids	327
Number killed in 4th instar	19
Intermediates with ocelli but no antennal sensoria	48
Intermediates with ocelli and antennal sensoria	247
Intermediates with patches of pigment on abdominal tergites	220

The development of the wing rudiments was followed through the various instars in several aphids which had become parasitized in the early second instar. At the time they were parasitized, there were no external signs of the wing buds. In the third instar, wing evaginations were formed but they were smaller than in normal third instar nymphs. In the fourth instar of some of the aphids, the evaginations appeared to be no more than exaggerated folds in the cuticle, and in the adult stage they were even less conspicuous. It was apparent that, from the second moult, the wingpads failed to differentiate any further and as the cuticle became stretched in each subsequent instar they became flattened out and were reduced or lost by the time the aphids reached the adult stage.

PARASITIZATION OF THIRD INSTAR ALATIFORM NYMPHS

Aphids parasitized in the third instar showed a considerable variation in the extent to which alatiform structures were suppressed when they became adult. They all had ocelli, antennal sensoria and wing rudiments, but the size of the wing rudiments and the degree of differentiation of the pterothorax varied according to the time during the instar that the aphids were parasitized and the number of parasite eggs that were deposited in them. All aphids that were parasitized in the third instar failed to develop pigment on the dorsal surface of the abdomen when they became adult, and most of them retained some of the nymphal pigmentation pattern of the head capsule.

Time of parasitization

A number of newly moulted third instar alatiform nymphs were collected and were divided into two batches; one batch was immediately placed in the parasite cage for three hours to be multi-parasitized and the other was put in the parasite cage for three hours on the following day. The aphids were kept at 20° C and, after moulting to the fourth instar, they were examined for effects of parasitization on development of the wingpads. The aphids which were parasitized at the beginning of the third instar all had smaller and more drooped wingpads than normal in the fourth instar; whereas the wingpads of the aphids parasitized later in the third instar were normal in the fourth instar. In both cases the wingpads failed to differentiate much further at the final moult (figs 10, 12).

Effect of single and multi-parasitization

One batch of newly moulted third instar nymphs was kept in the parasite cage for six hours to be multi-parasitized; in another batch the aphids were introduced individually to the parasites to have only one egg deposited in them. Both lots of aphids were then kept at 20° C and were examined when they moulted to the fourth instar. Only in the multi-parasitized aphids was there any effect on the wingpads in the fourth instar. When they became adults, all the multi-parasitized aphids ($n = 14$) had small rudimentary wingpads (fig. 10); of the single parasitized aphids, seven developed imperfect wings, about half the length of normal wings (fig. 12), and four had wingpads of the same size as those of fourth instar nymphs (fig. 11).

PARASITIZATION OF FOURTH INSTAR ALATIFORM NYMPHS

The effects on the structure of the adult cuticle caused by parasitization of fourth instar nymphs that were kept on healthy host plants were far less marked than the effects of earlier parasitization. No matter how early in the fourth instar they were parasitized or how many eggs were deposited in them, the aphids went on to develop distinctly alate structures, although these were sometimes imperfect.

The earlier in the instar the aphids were parasitized, the more extensive were the effects of parasitization. At 15° C, the fourth instar lasts for six days. A number of newly moulted fourth instar nymphs were collected and kept at 15° C and each day several of them were placed in the parasite cage for three hours. Aphids which were parasitized after the third day all became normal alate adults. In the aphids parasitized before the third day, the wings of several failed to expand fully; the antennae had typical apterous-type pigmentation; the tergites of all segments of the thorax failed to become fully sclerotized and pigmented; and in the mesothorax, the postnotum was not clearly differentiated from the notum.

As in third instar nymphs, multi-parasitization of fourth instar nymphs produced greater effects than single parasitization. Sixteen newly moulted fourth instar nymphs were oviposited in once, and sixteen were multi-parasitized. In the former group, there was only a slight thinning of the pigmentation along the median line of the postnotum and the prothorax; in the multi-parasitized aphids there were larger unpigmented and unsclerotized areas in these regions, and the wings were generally smaller than in normal alatae; in nine of them the wings failed to expand evenly and became deformed.

EFFECT OF RETARDING DEVELOPMENT OF THE HOST BY PARTIAL STARVATION

From the experiments described above it appeared that the extent of the effect of parasitization was related to the *amount* of parasite material inside the aphid at the time of formation of the new cuticle, and that this was a manifestation of both numbers of eggs present and the length of time the eggs had been in the host and consequently the amount of development they had undergone. If this were so, then by delaying moulting of the host by partial starvation and thus enabling the parasite eggs to attain a greater size before the host moulted, it should be possible to increase the effects of parasitization. Accordingly, a number of newly moulted fourth instar nymphs were put on a leaf in the parasite cage for six hours. The aphids were then removed from the leaf and for the next five days they were kept in a 20° C incubator, spending some of the time in a petri dish containing a wet filter paper, and some on an old, wilted host plant leaf. The duration of the fourth instar was extended in this way for two to three days. Out of an original 15 aphids, 5 died, 4 survived and were killed by the parasites while still in the fourth instar, and 6 moulted into adults. All the aphids which became adults had wing rudiments which were no larger than normal fourth instar wingpads; they had normal antennal sensoria, slightly reduced ocelli, no abdominal pigmentation, and the head capsule had a pattern of pigmentation that was more nymphal than adult in character (fig. 19). The extent of the suppression of general adult and alate structures was thus considerably increased by delaying the moult.

EFFECTS OF INJURIES ON THE DEVELOPMENT OF ALATIFORM STRUCTURES

The effects of parasitization became apparent in the aphid nymphs within a few days of the parasite egg being deposited in them and before the egg had actually hatched. It seemed possible that the effect may have been a response to injuries caused to the host by the parasite. Such injuries could have been caused by oviposition by the wasp or by toxic substances secreted by the wasp embryo.

Physical injuries were inflicted on aphid nymphs of different instars to ascertain whether injury alone could produce the effect of suppressing the development of alatiform structures. There was a high mortality of first instar nymphs which were pricked in the abdomen with a fine needle and, out of an original thirty-five which were injured, only nine survived: these all developed into apparently normal apterae, although in a control series of 362 aphids there were only 4% apterae. There had apparently been a complete reversal of form as a result of the treatment. Puncturing the abdomen of third instar nymphs had no effect on their subsequent development, but when a number of newly moulted third instar nymphs were given a severe burn injury to the abdomen with a microcautery, six out of eleven survivors developed into intermediates. They had normal alate ocelli and antennal sensoria but the pterothorax was imperfectly differentiated, the wings were only half expanded, and the antennae had apterous type pigmentation. Thus although the burn had been applied very early in the third instar, these aphids were very similar in structure to aphids which had been parasitized late in the third instar. However the dorsal surface of the abdomen of the burnt aphids was fully pigmented as in normal apterous aphids except for the site of the burn injury which was unpigmented, and the head pigmentation was adult in character. The burn injury thus affected only the alate characters and did not result, as did parasitization, in the retention of juvenile head and abdomen pigmentation. Severe injuries were also inflicted on fourth instar nymphs but in no case was the development of the normal alate structures interfered with.

SUPPRESSION OF DIFFERENT ALATIFORM STRUCTURES

1. *Ocelli*

In intermediates produced by the parasitization of aphids of different ages, the ocelli showed various degrees of suppression. In normal alate aphids there are three ocelli, the lateral ones situated immediately above the compound eyes and set back a short distance from the bases of the antennae and measuring about 25μ in diameter. In aphids with imperfect ocellar development, the lateral ocelli were moved forward and away from the compound eyes and were reduced in size (fig. 3). The smallest perfect ocellus found was only 5μ in diameter. Further stages in the suppression of the ocelli were found in some

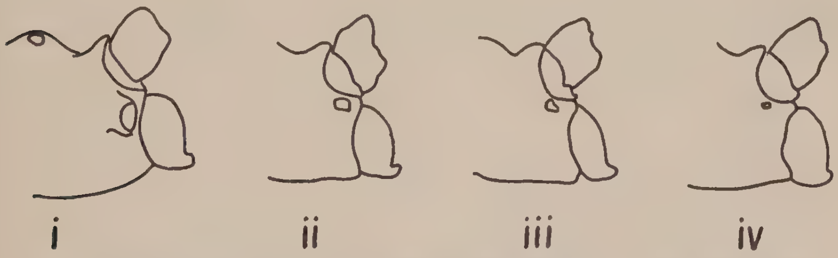


Fig. 3. Head, showing the size and position of the ocelli in i) normal alate adult; ii—iv) parasitized intermediates.

aphids which had been parasitized in the second instar: the ocelli were reduced to a raised ridge around a crater about 5μ in diameter but with no lens, in others slightly darker pigment in the vicinity of the ocelli was all that remained to indicate that the aphid was in fact an intermediate.

The median ocellus is situated in an awkward position for observation, and it could not be studied in as close detail as the lateral ocelli; however it was found in all the aphids with lateral ocelli which were examined.

2. *Antennal sensoria*

The placoid sensoria on the antennae showed various degrees of suppression in size and number in intermediates. The diameter of the sensoria was reduced in some intermediates that had been parasitized in the second instar, from 12-15μ to 5-6μ, and the number of sensoria on a single antenna was decreased from an average of five in the normal alate adult, through all intermediates to one or none (fig. 4). There was no apparent suppression of the sensoria in aphids parasitized in the third and fourth instars.

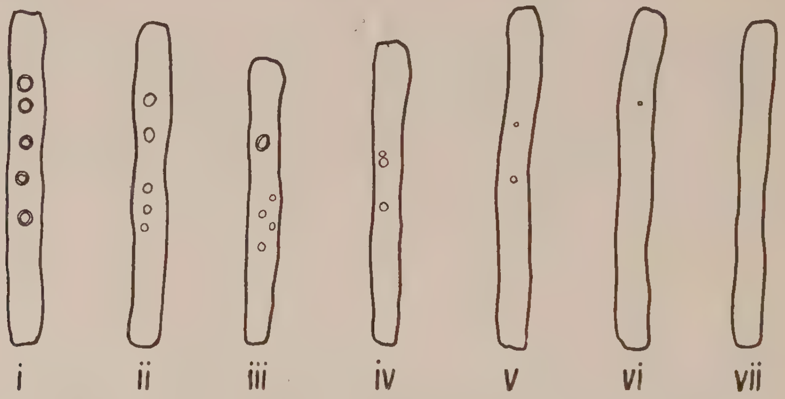


Fig. 4. Third antennal segment showing placoid sensoria in i) normal alate adult; ii—vii) parasitized intermediates.

The relative lengths of the third and fourth antennal segments of alate and

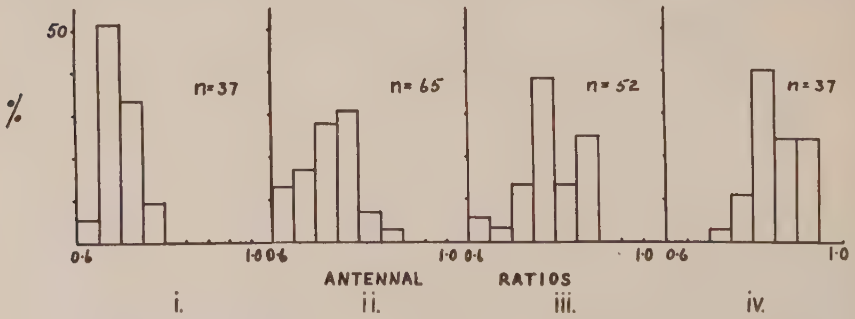


Fig. 5. Ratios of length of third to fourth antennal segments. i) normal apterous adults; ii) intermediates without antennal sensoria; iii) intermediates with antennal sensoria; iv) normal alate adults.

apterous aphids differ. In alatae, the sensoria-bearing third segment is shorter in relation to the fourth segment. In intermediate aphids, the antennal ratios were found to be intermediate between alatae and apterae: in intermediates without antennal sensoria (but with ocelli), the ratios tended to be closer to apterae, while in intermediates with antennal sensoria they tended to be closer to alatae (Fig. 5).

The fact that complete suppression of the antennal sensoria and the ocelli could be brought about by parasitization only in the second instar and not in later instars suggested that these structures become determined at that time, but do not go on to differentiate until the final moult. Their differentiation occurred at the final moult whether or not the aphid at that time was producing other alatiform structures. From this it might be argued that the differentiation of the antennal sensoria and ocelli is not necessarily controlled by the same mechanism as controls the differentiation of other alatiform structures. However it was possible to demonstrate that the antennal sensoria once determined (presumably in the second instar) go on to differentiate at the last moult regardless of whether they are then supplied by the moulting factor by an alatiform or an apteriform nymph. Antennae from newly moulted fourth instar alatiform and apteriform nymphs were implanted into one another and after the "host" aphid had moulted they were dissected out and examined. Sensoria always developed on antennae from alatiform nymphs but never on antennae from apteriform nymphs regardless of the form of the nymph into which they were implanted. It appears therefore that the differentiation of these sensoria is not dependent upon an alatiform type of humoral environment at the time of their formation as cuticular structures, although it does depend upon such an environment at the time of their determination in the second instar. The same appears to be true for ocelli, but to a lesser extent, as the ocelli, unlike the antennal sensoria, were considerably reduced in size in aphids that were parasitized in the third and fourth instars.

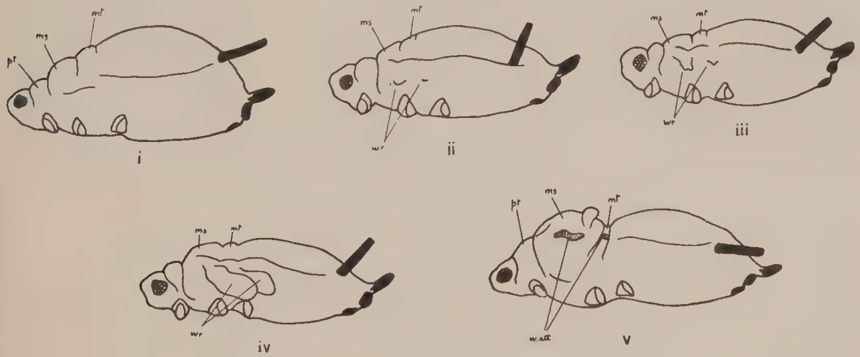


Fig. 6. Differentiation of the pterothorax. i) Normal apterous adult; ii) adult aphid which was parasitized in the second instar; iii) adult aphid multi-parasitized in early third instar; iv) adult aphid multi-parasitized in late third instar; v) normal alate aphid. pt: prothorax; ms: mesothorax; mt: metathorax; wr: wing rudiment; w att: wing attachment.

3. *Pterothorax*

A continuous series of degrees of differentiation of the thorax from the normal apterous thorax to the pterothorax of the alate was obtained in parasitized aphids, the extent of differentiation depending upon the age of the aphids at the time they were parasitized and consequently the extent of differentiation the pterothorax had already undergone, and whether one or many parasite eggs were deposited in them. In some aphids which had been parasitized in the second instar there was no trace at all of wingpads or other alatiform modification of the thorax, but later parasitization always resulted in intermediate pterothorax development (fig. 6).

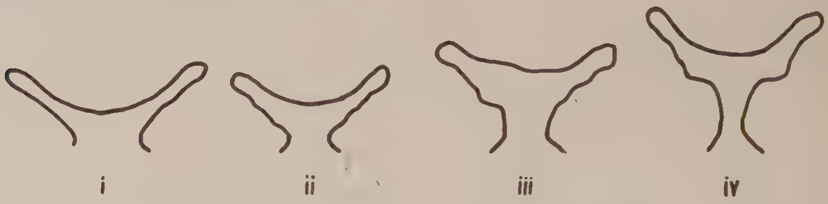


Fig. 7. Different degrees of development of the furca; i) apterous adult; ii, iii) parasitized intermediates; iv) alate adult.

Inside the thorax of intermediates, the flight muscles, the apodemes supporting them, and the furca were reduced corresponding to the degree of differentiation of the pterothorax (fig. 7).

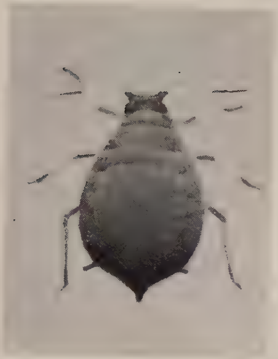
In describing intermediates of *Aphis fabae* Scop. which he found in laboratory cultures, DAVIDSON (1927) claimed to have observed wingpads inside aphids which had very small ocelli and antennal sensoria. In the present investigation, the aphids were carefully examined before and after clearing and before as well as after mounting on slides and no evidence was ever found of the presence of wingpads inside the thorax. Intermediates with a comparable development of the ocelli and antennal sensoria to that described by Davidson were all found to have normal external wing-rudiments. As Davidson examined only cleared mounted skins of the aphids, it seems probable that he was mistaken in believing the wing-rudiments to be inside the thorax.

4. *Other structures*

The genital plate is normally present only in adult aphids. Its differentiation was influenced by parasitization in two different ways. In aphids which were parasitized in the first instar, it was induced to differentiate prematurely in the fourth instar; and in aphids which were parasitized in the fourth instar and then starved, its differentiation was partly suppressed. In both cases the result was a cleft genital plate. As the cleft condition was produced both in nymphs showing prothetely and in adults showing metathetely, it would seem that the humoral conditions normally required for its differentiation probably lie between those required to produce the adult and those required to produce the juvenile form.

The dorsal surface of the head capsule is always completely sclerotized in adult alate aphids. Apterous adults, on the other hand, sometimes have small

B. Johnson : *Parasitization of* Aphis craccivora



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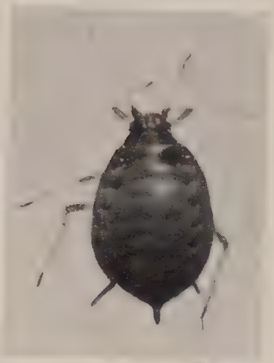

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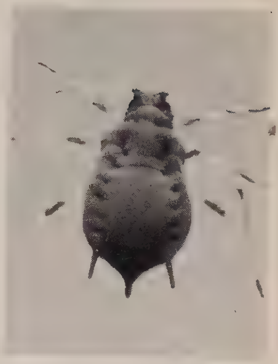

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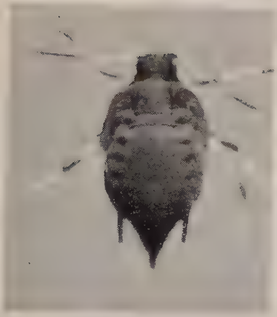

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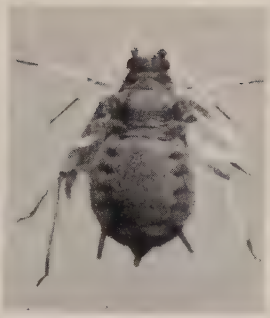

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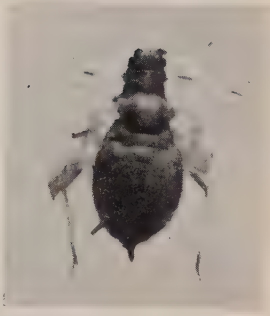

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Fig. 8. Fourth instar nymph which had become parasitized in the first instar. Except for having pigmented lateral muscle insertions on the abdomen and an intermediate shaped cauda, this aphid was identical to normal fourth instar apteriform nymphs. Fig. 9. Apterous adult produced from parasitization of second instar alatiform nymph. Pterothorax has failed to differentiate. Note pigmented dorsal surface of the abdomen. Fig. 10. Adult, multiparasitized early in the third instar. Note that there is no pigmentation of the dorsal surface of the abdomen. Fig. 11. Adult, single-parasitized early in the third instar. Fig. 12. Adult, multiparasitized late in the third instar. Fig. 13. Intermediate adult produced by giving early third instar nymph a severe burn on the abdomen.

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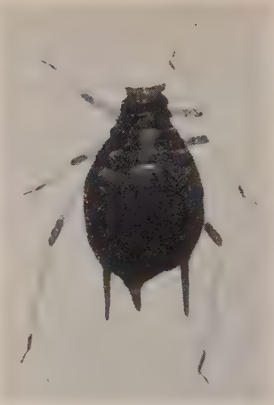

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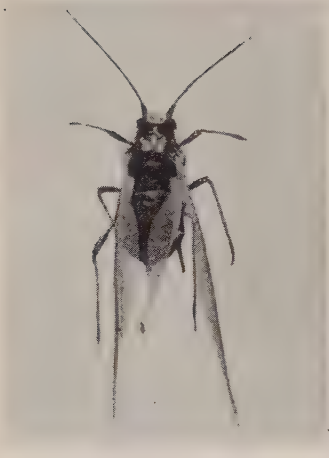

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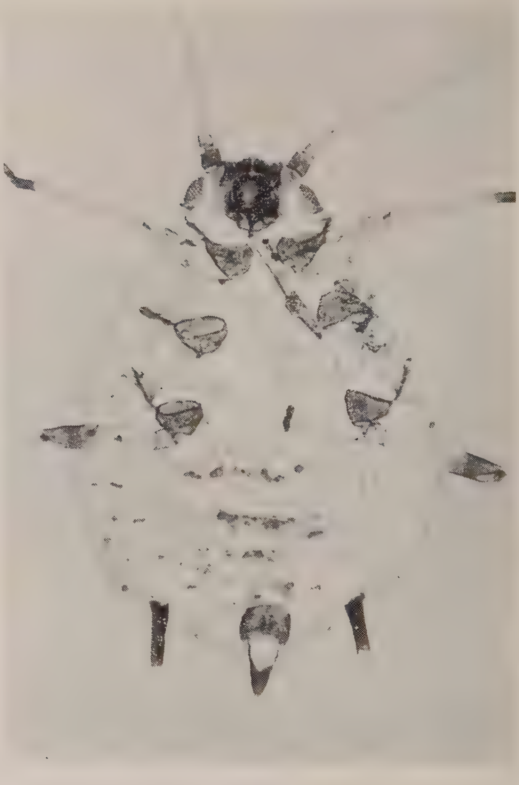

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Fig. 14. Normal fourth instar alatiform nymph. Fig. 15. Normal apterous adult. Fig. 16. Normal alate adult. Fig. 17. Cleared skin of fourth instar nymph which had become parasitized in the first instar and is showing prothetely. Note the intermediate-shaped cauda, cleft genital plate, and areas of pigmentation on the abdomen.

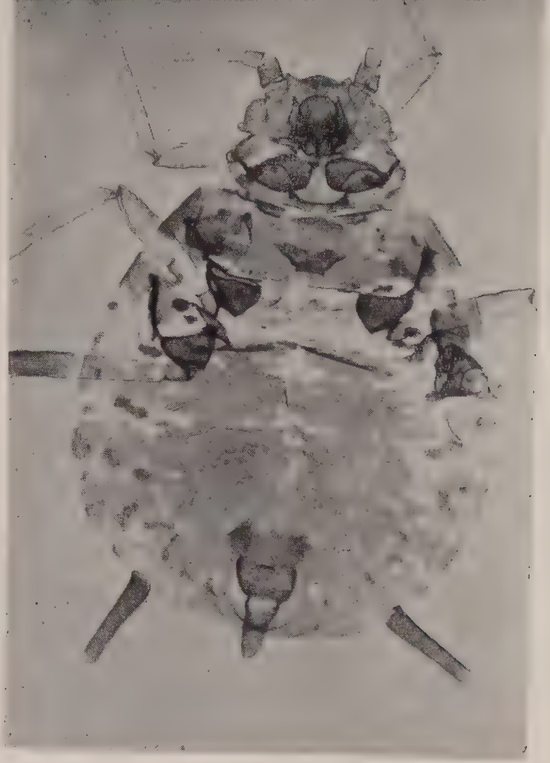

Fig. 18. Skin of adult which had become parasitized in the second instar. Note the pigmented abdomen and total suppression of wing development.

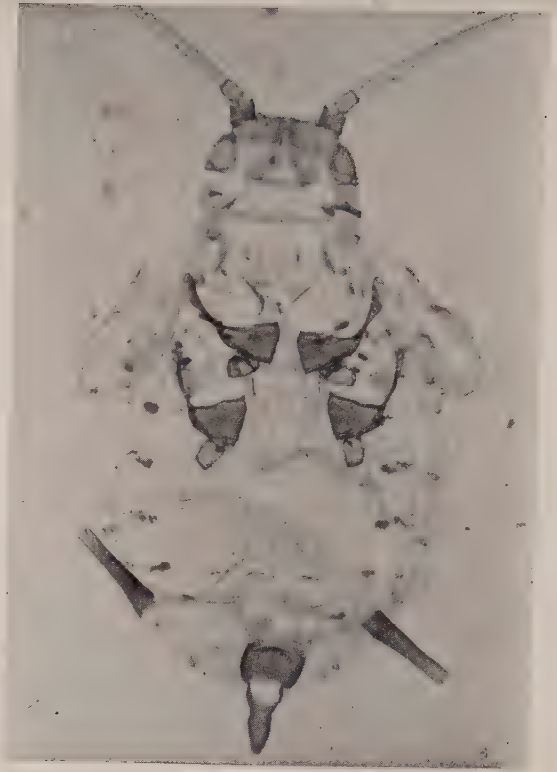

Fig. 19. Skin of adult which was multiparasitized in the early fourth instar and then starved to prolong the period before the final moult. The wingpads are no larger than those of fourth instar nymphs. Note the nymphal head pigmentation, absence of abdominal pigment, and cleft genital plate.

unpigmented areas behind the eyes and in the middle of the posterior margin of the head capsule. In *Brevicoryne brassicae* L. all stages of intermediates between the adult and the nymphal type of head capsule sclerotization are normally found in apterous adults; in normal *Aphis craccivora*, however, these unpigmented regions are frequently lacking and when they are present they are always very small. Adults of *A. craccivora* which had become parasitized in the second instar, and which therefore contained parasite larvae when they moulted into adults, all developed complete or almost complete adult type of head sclerotization; but aphids which had become parasitized in the third or fourth instars, and which therefore contained only unhatched parasite eggs at the time the adult cuticle was being formed, developed intermediate types of head capsules that were much more juvenile in character than is normally found in apterae of this species (fig. 19). Aphids which had become parasitized in the second instar also developed some dorsal abdominal pigmentation, whereas those parasitized later failed to develop any pigmentation of the abdomen.

DISCUSSION

A physiological interpretation of polymorphism in insects has been proposed by WIGGLESWORTH (1954). The epidermal cells are envisaged as having multipotentials with regard to the type of cuticle and cuticular structures they produce. Which of these potentials is realized depends upon the availability of a specific raw material, and this may be obtained directly from the food the animal ingests or it may be produced by an endocrine gland and circulate in the form of a hormone. Such a hormone is the juvenile hormone, or neotenin, which has been demonstrated in a number of insects and the presence and titre of which at the time of new cuticle formation influence the type of cuticle that is formed.

Polymorphism with respect to wing development occurs in a large number of different species of insects, but in none has the physiological factor which controls the extent of development of the wings been determined. Inadequate nutrition in males of *Gelis corruptor* (Grav.) was found by SALT (1952) to result in the production of apterous individuals, but whether the epidermal cells were reacting directly to a deficiency of a specific nutrient, or to modified hormone balance is not known. WIGGLESWORTH (1958) found that the application of juvenile hormone to the wing-rudiments of *Rhodnius* resulted in their failure to differentiate further. This and previous published accounts of the effect of juvenile hormone on wing differentiation (see WIGGLESWORTH, 1954) suggest that the various types of incomplete wing development that are found in a number of insects (aptery, microptery, brachyptery) could all be produced by upsets in the hormone balance of the insect in favour of the juvenile hormone. The extent of wing development that is found in the insects could be influenced by both the extent of upset of the hormone balance, and the stage of development of the insect at the time it is upset.

In aphids there are four different kinds of polymorphism. Besides the juvenile-adult and male-female forms that are found in most insects, there also occur apterous-alate and parthenogenetic-gamic forms. In *Aphis craccivora* sexual forms have not been found and this study is restricted to the agamic forms. KENNEDY (1956) drew attention to the dual attributes of adults of terrestrial animals generally: their possession of specialized sensori-motor apparatus and their ability to reproduce. Structurally, adult apterous aphids resemble young nymphs in that the sensori-motor apparatus as found in alate adults has failed to differentiate during their growth. In the sense that they do not possess these typically adult structures they are still juvenile, although they are adult in the sense that they are capable of reproduction and they possess several adult features such as head and abdomen pigmentation that are not obviously associated with either reproduction or locomotion. They may therefore be regarded as naturally occurring neotenics.

The results of parasitization of aphids described above support the theory that apterous-alate polymorphism in these insects is controlled by the same endocrine system as has been shown in other insects to control metamorphosis. The presence of parasite eggs in aphids resulted not only in the suppression of the development of the sensori-motor apparatus peculiar to alatae, but also in the retention of some nymphal characters, namely head and abdomen pigmentation. Metathetely was thus produced in a number of different structures, some of which were not only associated with alate adults. These effects of parasitization are very similar to effects produced in other insects by experimentally upsetting the hormone balance in favour of the juvenile hormone (WIGGLESWORTH, 1954); and it is suggested that such an upset is brought about in parasitized aphids.

If the hormone balance in aphids is upset by the presence of parasite eggs, how is this brought about? It could be caused by either the host modifying its own endocrine activity as a response to the presence of the parasite, or by the parasite directly influencing the titre of hormone in the blood of the host in much the same way as ichneumon parasites affect their syrphid hosts as demonstrated by SCHNEIDER (1950). It was found that injuries to alatiform aphid nymphs could result in their developing into apterae or intermediates instead of alatae: in young nymphs a complete switchover was effected, but in older nymphs it became increasingly difficult to interfere with the differentiation of alatiform structures, and other adult characters such as head pigmentation were not affected at all. Parasitization, however, was effective in suppressing general adult as well as specialized alatiform structures. Thus although it is possible that the effects of parasitization could in part be due to the presence of the parasite causing the host to modify its own endocrine activity, to account for the extensive and regular influence of parasitization, especially in late instar nymphs, on the differentiation of adult structures, it is difficult to escape the conclusion that the parasite egg directly affects the hormone balance in the blood of the host. The finding that the extent of the suppression of alate structures was affected by the number of parasite eggs that were laid, and the time that had elapsed between oviposition and moulting of the host, fits in well with this theory.

From the experiments in which aphids were parasitized at different times during the third and fourth instars it was evident that the parasites were exerting an effect on the host within a day or two of oviposition, although the eggs did not hatch until the third day. After oviposition and before hatching the eggs increase greatly in size and extensive cell division occurs as the embryos develop. NOVAK (1951) has shown that in late embryos of *Oncopeltus* the corpus allatum is actively producing juvenile hormone. It is suggested here that the developing embryos of *Aphidius* produce juvenile hormone and that some of it diffuses into the blood of the host, there increasing the titre and resulting in the observed effects on the cuticle. If this account is true, then production of juvenile hormone by the parasite embryo must occur well before differentiation of the corpus allatum, as until the third day after oviposition it is not possible to distinguish any differentiated structures in the embryo.

There is good evidence that the influence of the parasite that results in suppression of alate and other adult characters is exerted by the unhatched parasite embryo only, and not by the parasite larva. In aphids that were parasitized in the second instar, the differentiation of alatiform structures was inhibited, but at the final moult they developed typical adult head capsule and abdominal pigmentation, indicating that at the time of the last moult the parasite was not exerting so strong a juvenile influence as in aphids parasitized later in development, when these adult characters are suppressed by parasitization. It will be seen from fig. 1 that in aphids parasitized in the second instar the parasite egg hatches well before the initiation of endocrine activity preceding the final moult, whereas in aphids parasitized in later instars hatching occurs closer to or after this period. Further evidence that the juvenile influence not only ceases when the egg hatches but that after hatching the parasite larva can exert an opposite, namely adult, effect on the host is obtained from aphids parasitized in the first instar. These aphids developed prothetely as well as metathetely when they reached the fourth instar, the different effects being produced by exposure during their development to different conditions. During the first and second moults, they contained parasite eggs which were producing a juvenile influence and suppressing wing anlagen development, while at the third moult, they contained a parasite larva which was no longer exerting a juvenile influence but was possibly itself moulting and producing an adult influence by tipping the hormone balance in the opposite direction. The fact that at the third moult no alatiform structures developed was probably because for the production of alatiform structures the epidermis requires an alate-type humoral environment throughout development and not only at the last moult.

The failure of aphids that were parasitized in the first instar to develop any further than the fourth instar is worth comment. At the time that the aphids would be expected to complete their moult into the adult, the parasites were in the third instar, and while they occupied much of the abdomen of the host, they had not yet begun to attack any of the vital organs of the aphid, and would not have caused any physical damage to the host's endocrine organs. As a number of these nymphs developed prothetely, it is possible that the endocrine conditions in all aphids parasitized in the first instar may have been modified at the third moult sufficiently in the direction of the adult to have resulted in the thoracic glands responding to this moult as they normally would to the last moult, by breaking down. The thoracic glands of aphids have not yet been positively identified and this theory remains to be tested.

Although metathetely is known to be produced in a number of insects as a result of parasitization by various nematodes and protozoa, there appear to be no other instances (with the possible exception of Wheeler, 1910, quoted above) of its being caused by parasitization by an endoparasitic insect. The reasons for the apparent absence of this phenomenon in other insects are several, as are the conditions which must be fulfilled for it to occur. If the host insect is large, secretions from the tiny parasite eggs may become too diluted to be effective; the host insect must become parasitized in the juvenile stages and must then pass through at least one moult before the parasite egg hatches (aphids frequently pass through two moults); the effect is more likely to be produced by parasites which will deposit a number of eggs in the same host than by those which will oviposit in a host only once; and it is more likely to be found in hemimetabolous than holometabolous insects, and especially in those which exhibit different morphological forms of adults. There are probably very few host-parasite relationships which possess all these qualities.

Acknowledgements. I am indebted to Miss M. Szokoloczi for her assistance during this work and to Mr. K. P. Phillips for taking the photographs on the figures 8-16.

ZUSAMMENFASSUNG

DIE WIRKUNG DER PARASITIERUNG DURCH APHIDIUS PLATENSIS *BRÈTHES AUF DIE ENTWICKLUNGSPHYSIOLOGIE SEINES WIRTES,* APHIS CRACCIVORA *KOCH.*

Die normale Entwicklungsphysiologie der Junglarven von *Aphis craccivora* wird durch die Bestiftung mit Eiern von *Aphidius platensis* gehemmt. Dabei haben verschiedene Entwicklungsstadien des Parasiten gegensätzliche Wirkungen auf den Wirt: nichtgeschlüpfte Parasiteneier haben eine Verjüngungswirkung mit Metatelie als Endeffekt, während Junglarven des Parasiten manchmal eine verfrühte Ausbildung imaginaler Eigenschaften (Protelie) bei den Aphiden verursachen. Werden junge Nymphen des Wirtes parasitiert, so wird die kutikulare Differenzierung ihrer Flügelanlagen gehemmt. Daraus entstehen Imagines, die Intermediärformen zwischen geflügelten und ungeflügelten Morphen darstellen. Das Stadium der Differenzierung der kutikularen Strukturen bei der Belegung und die Anzahl der Parasiteneier bestimmen dabei den endgültigen Ausbildungsgrad dieser Anlagen.

Es wird angenommen, daß die Blattläuse metatelische Entwicklung zeigen, wenn ihr Juvenilhormontitre gestört wird, und daß der Flügelpolymorphismus durch das gleiche Hormonsystem kontrolliert wird wie Häutung und Metamorphose.

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DETERMINATION AU LABORATOIRE DE L'EPOQUE DE TRAITEMENT DE *TORTRIX VIRIDANA* L. AVEC UNE PREPARATION A BASE DE *BACILLUS THURINGIENSIS* BERLINER

PAR

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Une pulvérisation avec une préparation à base de *Bacillus thuringiensis* contre *Tortrix viridana,* effectuée au début de l'éclosion des oeufs reste pratiquement sans efficacité (test I). Une diminution sensible de la population larvaire est obtenue par une traitement au moment où la pénétration des chenilles néonates coïncide avec les stades réceptifs des bourgeons (test III).Quand les chenilles L 1 se trouvent à l'intérieur des bourgeons la réduction du niveau de population est nettement insuffisante. Les stades phénologiques du chêne correspondant au débourrement des bourgeons (V et VI) et qui concordent avec une population de jeunes chenilles, paraissent représenter la période la plus vulnérable à l'intervention microbienne. Cette période est de plus longue durée aux concentrations les plus élevées.

Les études d'application pratique de *B. thuringiensis* Berliner sont étroitement liées à la biologie des insectes. S'il est absolument indispensable d'entreprendre ces études dans la nature, afin de considérer l'insecte dans son milieu naturel, celles-ci peuvent être orientées efficacement par des études préliminaires au laboratoire où certaines conditions peuvent être artificiellement établies et contrôlées (GRISON, 1956; BURGERJON & GRISON, 1959; MARTOURET, 1959).

Les tests de laboratoire sur *Tortrix viridana* ont pu établir en fin février 1958, que les préparations de *B. thuringiensis* sont virulentes vis-à-vis des chenilles âgées (L5). Celles-ci provenaient d'un élevage précoce sur des rameaux de chêne naturellement contaminés, prélevés au mois de Janvier, puis forcés par trempage dans de l'eau tièdie au laboratoire.

De cette façon, nous avons obtenu dès le mois de février, de nombreuses chenilles qui furent d'ailleurs difficiles à nourrir car il n'y a pas de synchronisme entre le débourrement forcé du chêne au laboratoire et l'évolution rapide des chenilles. Néanmoins, l'utilisation de ces chenilles nous a utilement renseigné du point de vue des doses à choisir pour la série de tests effectuée ultérieurement en conditions plus naturelles.

L'étude plus précise de la sensibilité des divers stades de chenilles se heurte à la difficulté de prélever les jeunes chenilles à l'intérieur des bourgeons dans lesquels elles s'abritent pour se nourrir. Il en résulte un trouble dans la contamination par ces chenilles „dérangées" sur des rameaux traités au préalable: pour éviter les résultats trop artificiels qui auraient été obtenus à la suite des perturbations provoquées dans le comportement normal de ces chenilles, nous avons tenu compte des conditions de moeurs et de vie endophytes des jeunes

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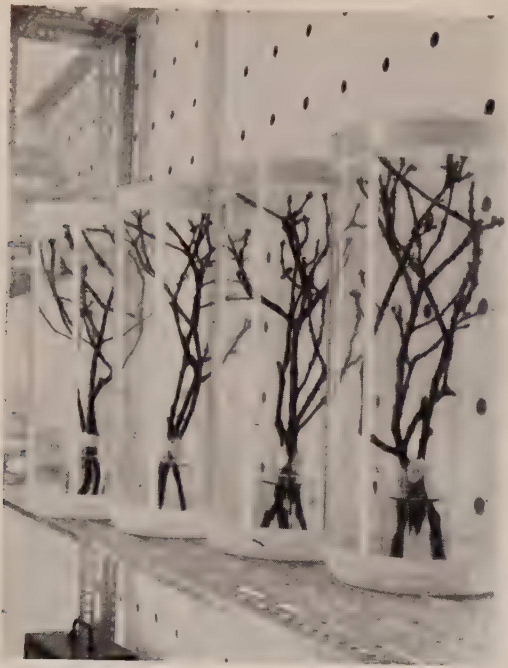


Fig. 1. Dispositif d'élevage en chambre climatisée (ici test no I).

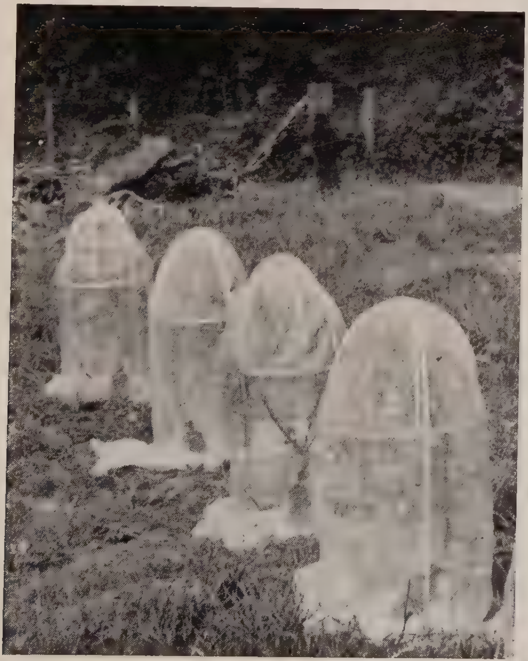


Fig. 2. Dispositif du test no VII.

chenilles en modifiant et adaptant à ces conditions la technique de nos tests de laboratoire sur *Tortrix viridana.*

METHODE EXPERIMENTALE

Huit essais consécutifs ont été réalisés afin d'établir l'efficacité d'un traitement en fonction des stades de bourgeons du chêne; ce qui a permis de déterminer au laboratoire l'époque de traitement la plus efficace pouvant orienter les tests et essais de traitement dans la nature. Ces tests consistaient à pulvériser la préparation bactérienne, en trois concentrations successives sur des rameaux prélevés au sommet d'un chêne *Quercus pedunculata* Ehrh. sans faire une infestation artificielle avec des chenilles. Nous nous étions assurés auparavant que l'arbre choisi était fortement infesté en oeufs. L'effet de la pulvérisation sur ces rameaux ne fut pas évalué par des pourcentages de mortalité, mais par la comparaison de la population survivante trouvée après une dizaine de jours sur les rameaux traités et sur les rameaux témoins.

Le premier test (I) a commencé le jour où les premières éclosions des oeufs furent constatées dans la nature. Ensuite sept autres tests furent faits sur des rameaux du même arbre à intervalle de deux à trois jours dans des conditions expérimentales identiques (excepté le test VII, qui fut réalisé en plein air, fig. 2). Ces conditions se résument ainsi: prélèvements des rameaux au même niveau de l'arbre (au sommet); longueur des rameaux = 35 cm portant environ 40 bourgeons pour chaque concentration; pulvérisation de 30 cc. de suspension (bouillie) dans notre tour de traitement (BURGERJON, 1956) quantité qui mouille abondamment les rameaux jusqu'au ruissellement; observation en chambre climatisée réglée à une température de 22° et 70 d'hygrométrie relative et en éclairage artificiel continu (fig. 1), dépouillement des rameaux et comptage de la densité de la population au moment où les chenilles des rameaux témoins quittaient ceux-ci par manque de nourriture (7 à 12 jours).

Matériellement, nous n'avions pas de possibilité de continuer l'élevage des survivants sur du feuillage non traité; cependant, par suite de l'effet toxémique pris en considération dans le titrage des préparations actuelles (BONNEFOI & BEGUIN, 1959) les données numériques représentées dans les figures jointes n'auraient pas été modifiées en valeur relative.

RESULTATS

Le tableau I montre les populations dénombrées les jours de dépouillement

TABLEAU I

Population de Tortrix viridana *denombrées les jours du dépouillement*

Tests	Date de traitements	Dépouillement		concentrations			
			Témoin	1	2	3	0
I	22/4/58	11 J. après	13	19	27	20	
II	24/4/58	11 „ „	15	23	6	5	
III	26/4/58	10 „ „	22	3	7	3	
IV	29/4/58	8 „ „	53	33	26	32	
V	2/5/58	8 „ „	23	1	3	2	
VI	5/5/58	7 „ „	21	0	0	0	
VII	7/5/58	12 „ „	99	17	6	9	
VIII	9/5/58	8 „ „	27	13	2	0	10

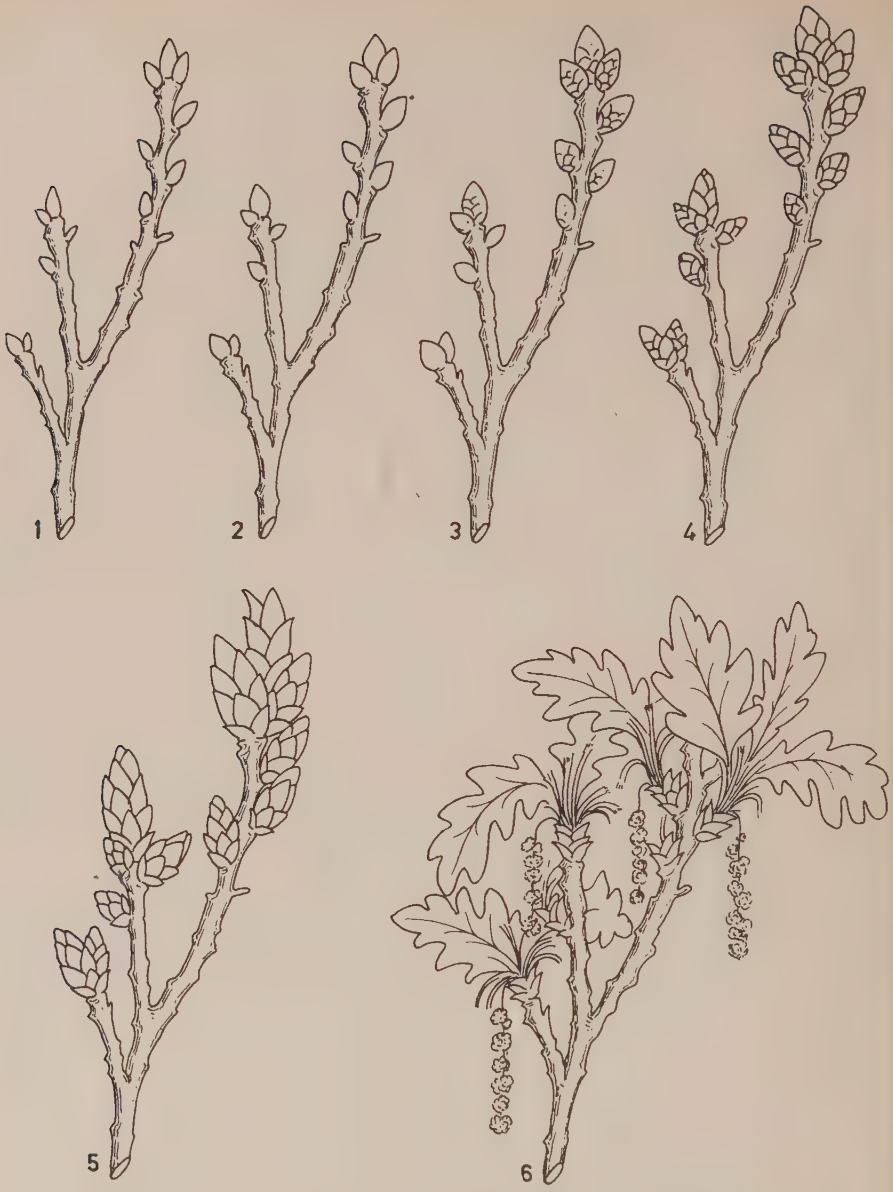


Fig. 3. Stades phénologiques des bourgeons, dessinés d'après photographies originales, chacun des no. I, II, III, IV, V et VI se rapportent aux tests correspondants.

Les concentrations indiquées par 0, 1, 2, 3 représentent respectivement 10, 20, 40 et 80 mgr. d'une poudre, titrant 900 U.B./mgr., diluée dans 30 cc. eau. Ces Unités Biologiques indiquant le degré de virulence des préparations provenant de l'Institut Pasteur, sont déterminées sur les chenilles de *Pieris brassicae* L. Les U.B. sont adoptées actuellement par notre Laboratoire et par l'Institut Pasteur, afin de s'assurer de la continuité d'un titrage normalisé (BONNEFOI, BURGERJON & GRISON, 1958; BURGERJON, 1957, 1959).

Les stades phénologiques des bourgeons sont caractérisés par la fig. 3 qui a été dessinée d'après les photos prises sur les rameaux même ayant subi les traitements. Nous avons essayé de comparer ces caractères photographiques aux stades des bourgeons décrits par SCHÜTTE (1957) (Tableau II).

TABLEAU II

Tableau des stades des bourgeons (d'après SCHÜTTE)

Tests		1 — Bourgeons entièrement fermés
I		2 — Bourgeons allongés (écailles pas encore détachées)
II		3 — Bourgeons tendres (un peu creux sous les écailles)
III		4 — Bourgeons ouverts (les écailles s'ouvrent à l'extrémité)
IV		5 — Bourgeons épanouis (souvent une feuille se déplie déjà)
V		6 — Pousse courbée - (plusieurs feuilles libres se déplient)
VI feuilles	2 à 4 cm	7 — Pousse étirée (des feuilles dépliées)
VII feuilles	6 à 7 cm	
VIII feuilles jusqu'	à 8 cm	

Dans la fig. 4a, le niveau de population survivante indique les proportions entre les divers stades de l'insecte, calculées sur une échelle relative de 100 pour faciliter la comparaison des tests entre eux. Pour chaque test l'échelle de 100 a été prise dans le lot contenant le plus grand nombre de chenilles survivantes.

A partir des données de la fig. 4a ont été travées les courbes de survie des fig. 4b et 4c. Celles-ci expriment la proportion de chenilles survivantes par rapport à celle du témoin. En réalité, ni l'une ni l'autre ne représente exactement l'effet des traitements, qui doit se trouver entre ces deux extrêmes. La première fait abstraction de la différence qualitative des stades des chenilles. Pourtant cette différence qualitative des stades exprime un retard d'évolution des chenilles survivantes, toujours bien caractéristique de l'intoxication des chenilles par *B. thuringiensis.* C'est en comparant les courbes de la fig. 4c avec celle de la fig. 4b que le retard d'évolution des chenilles survivantes apparait, puisque les courbes s'abaissent par rapport à celle du témoin et cet abaissement est plus marqué aux concentrations élevées.

DISCUSSION DES RESULTATS

Nous voyons sur les graphiques qu'à partir du test V les concentrations 2 et 3 sont très efficaces jusqu'à la fin de la série. Cette efficacité se prolonge certainement jusqu'au moment où les chenilles sont prêtes à se nymphoser (fig. 5). En effet, la concentration 2 fut retenue à la suite des tests précoces au laboratoire sur les chenilles en L5. La concentration 1 parait être suffisante à l'époque des tests V et VI. On peut expliquer ce fait par une meilleure mouillabilité de la suspension sur le feuillage qui commence à s'épanouir et par conséquent par une plus grande homogènéité de la pulvérisation.

Les époques représentées par les tests I, II, III et IV correspondent à une période qui débute par l'éclosion des oeufs et finit par la présence des chenilles

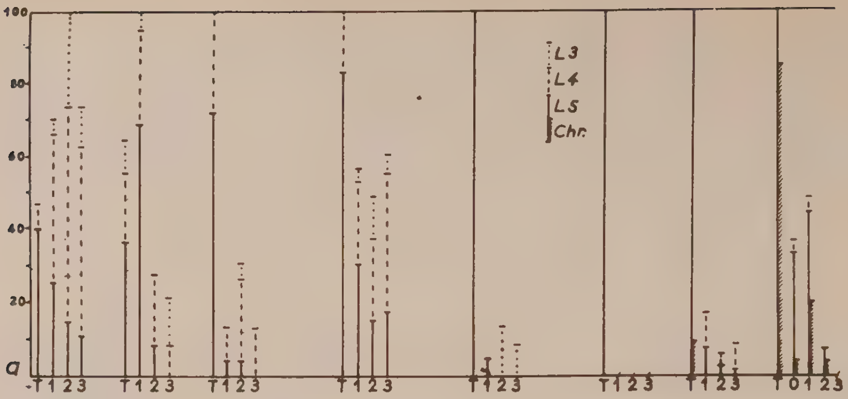


Fig. 4a. Répartition des stades de chaque population larvaire dans les essais au moment du dépouillement (100 correspond au nombre de chenilles du lot présentant le plus grand nombre de chenilles survivantes dans chaque test).

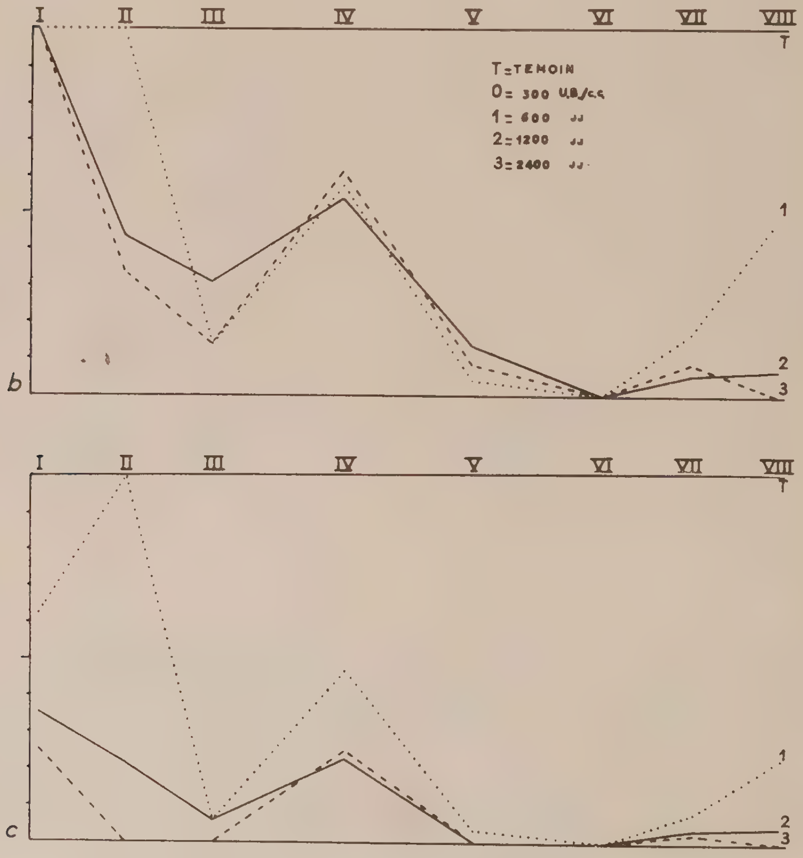


Fig. 4b et 4c. Courbes relatives de survie par rapport au témoin et en fonction des trois concentrations, établies au moment du dépouillement; en b: toutes les chenilles survivantes des stades L3, L4 et L5 sont totalisées; en c: seules les chenilles L5 sont comptées dans les tests I à VII, tandis que dans le test VIII seules les chrysalides sont comptées.

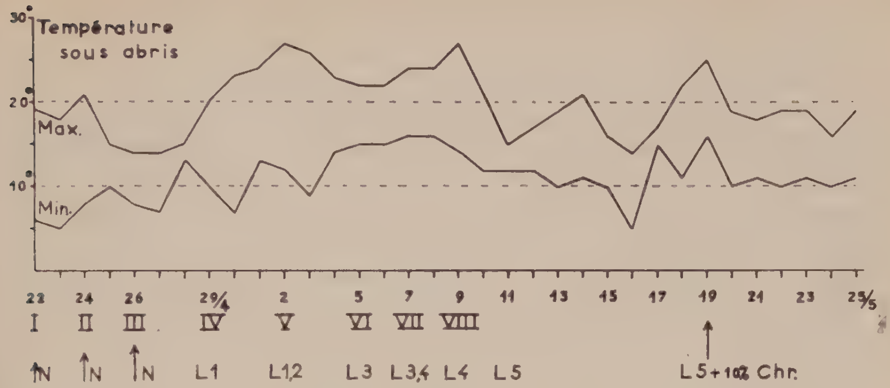


Fig. 5. Evolution des stades larvaires de *Tortrix viridana* L. dans la nature en 1958. La hauteur des flêches devant N représente les proportions de chenilles néonates balladeuses; les courbes de température expliquent la rapidité du développement des ler, 2è, 3è et 4è stades.

à l'intérieur des bourgeons, c'est-à-dire à une période de moindre vulnérabilité des insectes. Celle-ci s'atténue à l'époque du test III correspondant à la fois à l'éclosion massive des oeufs et au gonflement des bourgeons facilitant la pénétration immédiate des chenilles néonates, mais aussi la pénétration du produit. Au contraire aux époques du début d'éclosion des chenilles (tests I et II) les insectes s'alimentaient peu et attendaient le gonflement des bourgeons avant d'y pénétrer pour s'alimenter abondamment au dépens d'organes végétaux non recouverts de produit. Cependant on remarque quand même un effet aux concentrations 2 et 3 surtout à l'époque du test II.

Le test IV enfin représente la période où les chenilles LI se trouvent à l'intérieur des bourgeons sans mener de vie vagebonde, mais malgré celà il y a quand même une diminution des populations par rapport au témoin.

Les résultats des tests de laboratoire ne sont pas significatifs en ce qui concerne la protection des inflorescences et du feuillage, sauf pour les derniers tests. Pendant les premiers tests, il y a eu un déséquilibre artificiel entre l'évolution rapide des chenilles et le débourrement lent des rameaux du chêne privé de leur alimentation vasculaire; le feuillage était rapidement détruit par la voracité des quelques survivants de *Tortrix viridana* ainsi que de quelques autres espèces de lépidoptères.

Dans la nature, en 1957 et en 1958, la défeuillaison complète des arbres a été provoquée tardivement par les ravages des chenilles du 5éme stade. On peut donc s'attendre à ce qu'un traitement effectué aux époques favorables empêche la défeuillaison, puisque le traitement tardif dans le test VII permet d'obtenir un résultat satisfaisant à cet égard.

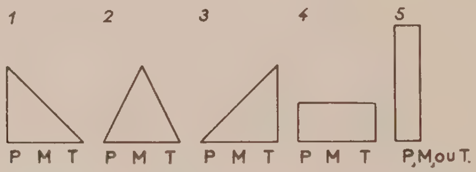
Le fait fondamental réside dans la très forte réduction de populations des chenilles jeunes.

INTERPRETATION DES RESULTATS DANS LA NATURE

Il ne sera pas prudent d'extrapoler sans discernement dans la nature les résultats obtenus au laboratoire; surtout en ce qui concerne les concentrations utilisées. Une température moyenne peu variable de 22° et un climat doux et sec au moment et après le test n° VII (fig. 5) ont favorisé une activité intense des chenilles. En effet, nous avons déjà montré à la suite de nombreux tests sur lépidoptères, que les facteurs qui conditionnent l'activité des chenilles, et en particulier la température, influencent l'efficacité de *B. thuringiensis.* En régle générale, il faut des doses plus élevées aux températures moins élevées (BURGERJON 1957).

Néanmoins, nous pensons que les résultats présentés ici peuvent être utiles pour la mise au point de traitements expérimentaux dans la nature avec *B. thuringiensis*, en tenant compte également des études de Schütte sur la dynamique des populations de *Tortrix viridana.* Ainsi il sera possible de choisir la meilleure époque d'intervention pour chaque peuplement de chêne phénologiquement défini.

Prenons quelques exemples extrêmes et théoriques de peuplements hétérogènes de chêne susceptibles d'être rencontrés dans lesquels nous distinguerons, selon la méthode Schütte, les arbres précoces (P.) moyens (M.) et tardifs (T.), soit quatre peuplements hétérogènes différents et un peuplement homogène dans lesquels la répartition relative des arbres selon leur précocité ou tardivité peut être représentée schématiquement dans les figures ci-contre/:



En 1. le peuplement est principalement constitué d'arbres précoces, le traitement pourrait être effectué quand les arbres moyens auront atteint les stades V et VI si l'écart entre P et T est très grand, et s'il y a eu une coïncidence entre l'éclosion des oeufs et la réceptivité des bourgeons sur les arbres P (voir Schütte). A ce moment les arbres T, peu nombreux, moins contaminés du fait de la non-coïncidence, et présentant des stades antérieurs à V peuvent recevoir néanmoins un traitement moyennement efficace.

En 2. le peuplement est principalement constitué d'arbres moyens en sorte que c'est le stade de ceux-ci qui sera déterminant pour la fixation de la date de l'application mais en attendant toutefois que les arbres T aient atteint le stade V.

En 3. où la majorité des arbres sont tardifs, on appliquera à peu près la même régle qu'en 4 où le peuplement est constitué en parties égales d'arbres P, M, et T, c'est-à-dire qu'on surveillera l'évolution des stades sur les arbres tardifs.

Dans le cas 5 d'un peuplement homogène en arbres P, M, ou T, il sera facile de déterminer la date d'application aux époques V et VI en utilisant une dose relativement faible par rapport aux cas précédents.

On voit donc par ces quelques exemples, qu'il importe avant tout de connaître:

1 — *La population* en arbres précoces, moyens et tardifs du peuplement.
2 — *L'amplitude* des écarts phénologiques de ces groupes d'arbres.
3 — Avec quel groupe d'arbres il y a *coïncidence* vis-à-vis de l'éclosion des oeufs.
4 — *La densité de la population* et la détermination du *„seuil de nuisibilité"*, afin d'éviter des traitements inutiles.

On peut distinguer deux „seuils de nuisibilité" dans le cas de *Tortrix viridana*. Premièrement, une population initiale cause la défeuillaison à la suite du ravage des L5. Deuxièmement, il peut se présenter occasionnellement une très grande population aux stades des chenilles néonates, ce qui détermine des dégâts maximum aux stades des bourgeons et des jeunes pousses. Dans la première éventualité l'intervention bactérienne aux stades V et VI est du type „préventive" et doit permettre d'éviter la défeuillaison; dans la deuxième, elle est plutôt du type „curative" et aura pour objet essentiel la destruction du plus grand nombre possible de chenilles, en sorte qu'une intervention au stade du test III pourrait être complétée par un deuxième traitement aux stades V et VI.

Les méthodes permettant de connaître ces seuils de nuisibilité ainsi que les méthodes qui permettent de prévoir ceux-ci devront faire l'object d'une étude détaillée, où les études de Schütte formeront déjà des données de départ.

Enfin, nous devons compléter ces données phénologiques par des remarques biocoenotiques.

L'effet d'un traitement dans la nature de *B. thuringiensis* contre la tordeuse verte devra être évalué non seulement l'année de l'application, mais également les années suivantes. La non-toxicité de *B. thuringiensis* vis-à-vis des parasites et prédateurs entomophages assurera la survie d'une partie non négligeable de la faune entomophage (BILIOTTI, 1956). Par conséquent, après une application efficace, il n'est pas illusoire de supposer que le taux de parasitisme sera plus élevé sur la faible population survivante, au moins en ce qui concerne les parasites qui cherchent „activement" leur hôte.

En Espagne, où les dégâts causés par *Tortrix viridana* sur le chêne vert *Quercus ilex* sont énormes (TORRENT 1955, 1958), l'intervention à l'aide d'insecticides chimiques doit provoquer des hécatombes de parasites et doit être l'une des raisons du renouvellement annuel de ces traitements.

Il serait intéressant d'étudier spécialement l'incidence sur la biocénose des traitements bactériens. Nos observations dans cette matière sont encore très sommaires et mériteraient d'être reprises et complètées méthodiquement.

CONCLUSIONS

Les tests de *B. thuringiensis* sur *Tortrix viridana* avec les préparations bactériennes actuellement présentées sous forme de poudre de spores et de cristaux permettent d'envisager contre ce ravageur une lutte beaucoup plus rationnelle qu'auparavant. Cette méthode de lutte est prometteuse puisque, sans connaître encore en détail les répercussions biocoenotiques, les préparations à base de

B. thuringiensis se distinguent fondamentalement par leur „sélectivité", de la plupart des insecticides chimiques.

La lutte contre *Tortrix viridana*, sera conduite d'autant plus rationnellement dans chacun des biotopes spéciaux de l'insecte, que l'intervention microbiologique tiendra compte à la fois des données des études de la biocénotique et de celles de la dynamique des populations.

Notre travail a pu démontrer que sur un arbre déterminé, l'on peut choisir la meilleure époque d'intervention en se basant simultanèment sur la phénologie de l'arbre et sur celle de l'insecte; dans un peuplement hétérogène de chênes, le choix de la meilleure époque d'intervention doit en outre tenir compte de l'ensemble des facteurs variables du biotope.

ZUSAMMENFASSUNG

LABORMÄSSIGE BESTIMMUNG DES ZEITPUNKTES ZUR BEHANDLUNG VON TORTRIX VIRIDANA *L. MIT AUFSCHWEMMUNGEN VON* BACILLUS THURINGIENSIS *BERLINER*

Der günstigste Termin einer Behandlung der Raupen des Eichenwicklers (*Tortrix viridana* L.) mit *Bacillus thuringiensis* Berliner wurde durch acht aufeinanderfolgende Versuche ermittelt. Im Moment des Ausschlüpfens der Eier in der Natur wurden Zweige, deren Knospen noch nicht aufgebrochen waren, abgeschnitten. Die Wirkung der an diesen Zweigen in drei verschiedenen Konzentrationen durchgeführten Spritzbehandlungen wurden nicht in Prozenten der Sterblichkeit, sondern durch Vergleich der Populationen der behandelten und der unbehandelten Zweige ermittelt. Diese Kontrolle wurde ungefähr 10 Tage nach der Behandlung durchgeführt.

Auf dem gleichen Baum wurden in Zeitintervallen von 2—3 Tagen sieben weitere Versuche unter identischen Bedingungen durchgeführt. Die Wirksamkeit wurde in einer Kurve dargestellt, welche den direkten Zusammenhang mit dem jeweiligen Knospenstadium erkennen läßt. Um dieses Stadium festzuhalten, wurde bei jedem Versuch ein Photo der Versuchszweige gemacht. Die Kontrolle verschiedener Zweige desselben Baumes am Tage der Behandlung gestattete, das Stadium der in den Knospen und eingerollten Blättern gefundenen Insekten zu bestimmen.

Eine Behandlung zu Beginn der Schlüpfzeit bleibt beinahe ohne jede Wirkung (Versuch I). Die Behandlung während der kurzen Periode, während der sich die jungen Raupen im Inneren der Knospen aufhalten, zeigte ebenfalls keine genügende Wirkung (Versuch IV). Eine stärkere Verminderung der Population konnte jedoch festgestellt werden, wenn die Behandlung während der kurzen Periode des massiven Ausschlüpfens durchgeführt wurde (Versuch III). Der günstigste Termin für die Behandlung beginnt mit dem Aufbrechen der Knospen (Fig. 3V) und dauert an, bis die Blätter sich gänzlich entfaltet haben (Versuch VI). Sobald die Blätter vollständig entwickelt waren, wurden nur noch mit den höchsten Konzentrationen gute Resultate erzielt (Versuch VIII).

Die Resultate dieser Versuche, die auf einem bestimmten Baume durchgeführt wurden, sind nur von orientierendem Charakter. Um Versuche im Freiland planmäßig durchzuführen, muß der ungleichen Entwicklung der Eichen und der Koinzidenz zwischen Eiche und Wickler Rechnung getragen werden, außerdem muß die Schädlichkeitsschwelle des Eichenwicklers bekannt sein.

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EXPERIMENTS WITH SUCTION TRAPS TO ASSESS THE ABUNDANCE OF SYRPHIDAE (DIPTERA), WITH SPECIAL REFERENCE TO APHIDOPHAGOUS SPECIES

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Preliminary information on the abundance of some common species of Syrphidae was obtained from the catches in five suction traps, operated at Rothamsted during the summers of 1951, 1952 and 1953. Six of the eight "abundant" species were aphidophagous.

The numbers of the individual species recorded varied from year to year, with the time of year, and from place to place.

Although the catches could not be correlated with the immediate weather conditions, the large differences between them could be associated with the differences in the general weather conditions of the three years. Syrphidae were few in the spring, although most species showed a small peak of numbers then, and maximum catches of nearly all species were recorded in late summer (August).

At two different localities, there were conspicuous differences in the abundance of individual species and in the composition of the catches. Where the vegetation was varied, Syrphidae were more diverse and more abundant than where the vegetation was uniform. Some species occurred in one locality but not in the other. The size and composition of the catch were not obviously affected by the kind of vegetation immediately around a trap.

Some of the many factors affecting the abundance of Syrphidae are discussed.

Although many Syrphidae are beneficial to man because their larvae eat aphids, little has been published on changes in abundance of individual species throughout the years, or from year to year. Species have been described in such terms as "common", "uncommon" or "rare", but little has been recorded on the relative abundance of even the commonest species. From collections of adult Syrphidae and by rearing adults from larvae, SCHNEIDER (1948) recorded the monthly abundance of several Swiss species, some of which occur also in this country.

Syrphidae have occasionally been reported in large numbers (BANKS, 1952; COE, 1953), and some species are known to migrate (ELTON, 1925; MACKWORTH-PRAED, 1929; WILLIAMS, 1958), but the migrations of Syrphidae have not been studied so closely as those of Lepidoptera and other insect groups. SCHNEIDER (1958) described the daily movements of *Scaeva pyrastri* (L.) in two localities in Switzerland. The insects fly long distances, descending 600 m or more from their overwintering and night-time quarters in spruce forests on the mountains to the valleys, where they feed on the pollen of various plants.

To investigate the abundance of Syrphidae, experiments with five suction traps were made at Rothamsted during the summers of 1951, 1952 and 1953, as part of a study of the predator complex of *Aphis fabae* Scop., the Black Bean Aphid; my interest was centred, therefore, on those species of Syrphidae whose larvae are aphidophagous.

The suction traps (JOHNSON, 1950) used in the experiments sample a very small quantity of air and, unlike light traps operating at night, do not attract insects from a distance; only those insects flying over the traps are captured. The traps were, however, identical and it is permissible to compare the catches of individual species and to gain some idea of the relative abundance of different species.

Syrphidae are very active diurnal insects which move about almost entirely by flying. Some of the larger species, such as *Scaeva* spp. and *Syrphus ribesii* (L.), are strong fliers and may escape capture in small suction traps; in all five traps, only one specimen of *Scaeva* (*S. selenitica* (Meigen)) was captured, although its larvae occurred on the bean plants. Other species, such as the smaller *Platycheirus*, are often seen flying close to the ground and they, too, may escape

TABLE I

Duration of periods of trapping

Year:			1951	1952	1952	1953	1953
Vegetation:			beans	beans	beans	beans	weeds
Trap No:			1	2	3	4	5
Week ending		Week No.					
April	16	1		│		│	│
	23	2		│		│	│
	30	3		│		│	│
May	7	4		│		│	│
	14	5		│		│	│
	21	6		│		│	│
	28	7	│	│	│	│	│
June	4	8	│	│	│	│	│
	11	9	│	│	│	│	│
	18	10	│	│	│	│	│
	25	11	│	│	│	│	│
July	2	12	│	│	│	│	│
	9	13	│	│	│	│	│
	16	14	│	│	│	│	│
	23	15	│	│	│	│	│
	30	16	│	│	│	│	│
August	6	17	│	│	│	│	│
	13	18	│	│	│	│	│
	20	19	│	│	│	│	│
	27	20	│	│	│	│	│
September	3	21	│	│	│	│	│
	10	22	│	│	│	│	│
	17	23		│	│	│	│
	24	24		│	│	│	│
October	1	25			│	│	│
	8	26			│	│	│
	15	27				│	│
	22	28				│	│
	29	29				│	│
November	5	30				│	│
	12	31				│	│
	19	32				│	│
	26	33				│	│

capture. The traps may not, therefore, give a fully representative picture of all the Syrphidae in a locality and this possibility must be kept in mind when interpreting the results.

The first experiment was designed to study the changes in abundance of adult Syrphidae during the three successive summers at one small locality where the vegetation was similar from year to year. The second was to compare the catches of these flies at the same kind of vegetation (field beans, *Vicia faba*), but at two different localities. The third experiment was to compare the catches at two different kinds of vegetation in the same small area.

METHODS

Suction traps with fans of 9 in. (22.8 cm) diameter, which sample approximately 300 cu. ft. (8.5 cu. m) of air per minute (Taylor, 1955), were used without an automatic segregating mechanism (Johnson, 1950). They ran continuously during the periods shown in Table I, and were emptied of insects every day, except in the early and late weeks of the trapping periods when they were emptied every two or three days. The daily catches of Syrphidae were totalled for each week. The weeks, for convenience, have been numbered from an arbitrary starting date, 10th April, in each year.

The plots of spring-sown field beans *(Vicia faba)* consisted of twelve rows, each 35 ft (10.7 m) long and spaced 30 in. (76 cm) apart. Traps 1, 2 and 4 (1951, 1952 and 1953 respectively) were sited at Rothamsted Lodge, the garden of which is surrounded by tall trees and buildings and on one side of which is a high brick wall, so that the locality is fairly isolated and secluded. The garden contained a great variety of plants and trees, including many flowering plants, fruit trees and various vegetables, but the vegetation was similar throughout the three years. Trap 5 in 1953 was also in the garden, 30 ft (9 m) away from trap 4, in the middle of a small plot of weeds of about the same area as the bean plot adjoining it. The plants there included nettles (*Urtica dioica* L.), mugwort (*Artemisia vulgaris* L.), scentless mayweed (*Matricaria inodora* L.), greater plantain (*Plantago major* L.), *Chrysanthemum* sp., and other plants.

Trap 3 (1952) was sited one quarter of a mile (404 m) from Rothamsted Lodge in the middle of a plot of spring-sown field beans. The vegetation surrounding this plot, large fields of wheat, beans and potatoes, was less diverse than that at the Lodge garden and the plot was more exposed to the wind and to immigrant Syrphidae and other insects. The traps were supported on frames so that the mouths were 3 ft (0.9 m) above ground and level with the tops of the plants.

Traps 2 and 3 are identical with traps *a* and *b* of an earlier paper on Coccinellidae (Banks, 1955) and traps 4 and 5 (only) are identical with traps 4 and 5 of Southwood (*in litt.*).

Of the very few syrphids damaged in the trapping most were still identifiable. The nomenclature used in this paper is that of Parmenter (1954), which is based on Coe (1953).

THE RELATIVE ABUNDANCE OF THE SPECIES CAUGHT IN THE TRAPS

COE (1953) lists 97 species of British Syrphidae whose larvae are known to be, or are thought to be, predators of aphids. Of the 54 of these species which he calls "common" or "frequent", 32 were recorded in the five suction traps during the three summers; 22 common or frequent aphidophagous species

TABLE II

Totals of aphidophagous and non-aphidophagous Syrphidae in the five traps

Year:	1951	1952	1952	1953	1953	
Trap No:	1	2	3	4	5	Totals
Melanostoma mellinum (Linnaeus)	123	60	52	133	164	532
Platycheirus albimanus (Fabricius)	94	91	10	61	53	309
Platycheirus clypeatus (Meigen)	98	17	33	51	45	244
Syrphus balteatus (Degeer)	59	28	3	75	71	236
Syrphus vitripennis Meigen	63	5	0	24	27	119
Platycheirus scutatus (Meigen)	21	20	2	19	38	100
Melanostoma scalare (Fabricius)	14	19	7	4	14	58
Syrphus corollae (Fabricius)	1	8	27	0	5	41
Platycheirus peltatus (Meigen)	9	17	3	2	5	36
Syrphus auricollis Meigen	10	7	1	2	4	24
Platycheirus manicatus (Meigen)	0	8	3	3	8	22
Syrphus ribesii (Linnaeus)	6	5	1	3	4	19
Syrphus albostriatus (Fallen)	0	4	2	0	0	6
Syrphus luniger Meigen	0	3	2	1	0	6
Syrphus lunulatus Meigen	0	2	0	0	0	2
Syrphus cinctus (Fallen)	0	1	0	0	0	1
Syrphus venustus Meigen	3	1	0	0	0	4
Syrphus umbellatarum (Fabricius)	0	1	0	0	0	1
Syrphus torvus Osten-Sacken	0	1	0	0	0	1
Syrphus latifasciatus Macquart	0	1	0	0	0	1
Syrphus eligans (Harris)	1	0	0	0	0	1
Sphaerophoria menthastri (Linnaeus)	0	2	1	0	0	3
Sphaerophoria scripta (Linnaeus)	0	0	0	0	1	1
Pipiza noctiluca (Linnaeus)	1	0	0	0	1	2
Pipiza luteitarsis Zetterstedt	0	1	0	0	0	1
Heringia heringii (Zetterstedt)	0	1	0	0	0	1
Baccha elongata (Fabricius)	2	1	1	2	0	6
Baccha obscuripennis Meigen	2	0	0	0	0	2
Scaeva selenitica (Meigen)	0	0	0	0	1	1
Platycheirus angustatus (Zetterstedt)	1	0	0	0	0	1
Pipizella sp.	0	1	0	0	0	1
Cnemodon sp.	0	1	0	0	0	1
Total individuals	508	306	148	380	441	
Total species	17	26	15	13	15	
Index of diversity	3.5	7.0	4.0	2.8	3.2	
error (%)	12	12	16	14	13	
Total aphidophagous species						1783
Syritta pipiens (Linnaeus)	131	233	25	74	72	535
Neoascia spp.	66	74	24	39	25	228
Others (11 spp.)	10	19	0	10	26	65
Total non-aphidophagous species						828
Grand total						2611

were not caught. The remainder Coe classifies as "uncommon" or "rare". Aphidophagous species among the following genera were not trapped: *Paragus, Pyrophaena, Xanthandrus, Xanthogramma, Didea* and *Triglyphus.*

Of the 32 aphidophagous species, Coe calls 29 of them "common" or "frequent". Three species which were trapped, *Pipiza luteitarsis* Zetterstedt, *Scaeva selenitica* (Meigen) and *Heringia heringii* (Zetterstedt), he calls "uncommon" and they were scarce in collections. Six of the aphidophagous species, whose numbers totalled 100 or more, were much more numerous than any of the others and I have called these "abundant"; together they formed 86 per cent of all the aphidophagous forms. They were *Melanostoma mellinum* (L.), *Platycheirus albimanus* (Fabricius), *P. clypeatus* (Meigen), *Syrphus balteatus* (Degeer), *S. vitripennis* Meigen and *P. scutatus* (Meigen), and were, with one exception, the six most abundant species in each trap (Table II); in trap 3, *S. balteatus* was very scarce and *S. corollae* (Fabricius) was more abundant in its place (see below). Table III lists these species, which with the two abundant non-aphidophagous forms, *Syritta pipiens* (L.) and *Neoascia* spp., formed 88 per cent of the 2611 individuals caught in all the traps. Other common species given in Table I were not abundant.

Melanostoma mellinum was nearly always more abundant than any other aphidophagous species (Table II & III) and was about 10 times as abundant as the other common species of the genus, *M. scalare* (Fabricius). *M. mellinum* alone accounted for 30 per cent of all the aphidophagous species and, with *P. albimanus,* was nearly half the total of these forms. *S. corollae* and *S. ribesii,* both common insects, were not very numerous in the collections and were, in fact, less numerous than the "frequent" *S. vitripennis. Syritta pipiens* was as abundant as *M. mellinum,* but was more numerous in the early summer and less abundant in late summer. *Neoascia* spp. were also fairly abundant but other non-aphidophagous species were scarce.

TABLE III

Monthly totals of the most abundant Syrphidae in the five traps

	M. mellinum	*P. albimanus*	*P. clypeatus*	*S. balteatus*	*S. vitripennis*	*P. scutatus*	*M. scalare*	*S. corollae*	*P. peltatus*	*S. auricollis*	*P. manicatus*	*S. ribesii*	Totals	*S. pipiens*	*Neoascia* spp.	Totals
April	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	6
May	9	28	3	0	0	9	7	0	0	0	3	1	60	59	43	102
June	9	20	13	2	3	12	0	5	11	1	1	2	79	90	9	99
July	132	73	47	35	14	8	17	31	5	8	4	4	378	161	64	225
August	320	148	166	168	82	50	19	5	12	13	6	12	1001	142	67	209
September	60	40	15	30	20	20	15	0	8	2	8	0	218	81	39	120
October	2	0	0	1	0	1	0	0	0	0	0	0	4	2	0	2
November	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Totals	532	309	244	236	119	100	58	41	36	24	22	19	1740	535	228	763

Although Syrphidae are seen flying in the spring, none was caught in the traps during April and the first captures were not made until early May (Table III).

The insects were most abundant in August and, thereafter, numbers declined rapidly. Few occurred in October and none was caught during November and December.

EXPERIMENT I. *Comparison of captures of Syrphidae at a bean plot at Rothamsted Lodge in 1951, 1952 and 1953*

A trap was operated in a crop of spring-sown field beans on the same small plot of land at Rothamsted Lodge during the summers of 1951, 1952 and 1953. The adult Syrphidae caught there are compared for weeks 7-22 (22 May — 10 September) in each year.

The six abundant aphidophagous species (Table IV) were 107 per cent more numerous in 1951 than in 1952, but only 26 per cent more numerous in 1951 than in 1953; the numbers of 1953 were 63 per cent higher than those of 1952. A comparison of the index of diversity (WILLIAMS, 1947) for each collection at Rothamsted Lodge garden shows (Table II) that the catches for 1951 and 1953 (traps 1 and 4) did not differ significantly from each other, but both differed significantly from the catch for 1952 (trap 2). In 1952, the more common species were fewer and the less common species were more numerous than in the other two years. In contrast, the non-aphidophagous species, *Syritta pipiens,* was most abundant during 1952, half as abundant in 1951 and scarce in 1953.

Maximum numbers of nearly all species were recorded in late July or in August. Table IV shows that in 1952 the captures of all species and the time of their individual maxima occurred earlier in the season than in the other years. During 1953, the maximum catches occurred comparatively late, and most Syrphidae were caught during a short period of a few weeks; whereas in 1951 and 1952, 75 per cent of all the insects were caught within 10 or 11 weeks of the capture of the first syrphid, in 1953 75 per cent of all species were trapped within an average time of about 6 weeks. There were many weeks during the spring and summer of 1953 when no Syrphidae were caught; in fact, no aphidophagous syrphid was caught before week 13 (3—9 July) in that year.

These variations can be associated with the annual weather conditions. The main peak of captures in 1952 was preceded by a smaller peak in May; syrphids were captured earlier in 1952 presumably because of the warm, early spring when adult Syrphidae, which had developed from overwintered larvae, emerged early. The figures for 1951 indicate a similar but not so pronounced peak in the spring. In contrast, the spring and early summer of 1953 were cold and wet and Syrphidae and other insects were scarce for a long period. The Syrphidae became abundant in the trap only when the weather improved in August.

Platycheirus species were most abundant in 1951; only three of them can be considered numerous. *P. albimanus* was the most abundant in all years; *P. clypeatus* was scarce in 1952 but was as abundant as *P. albimanus* in the other years. *P. scutatus* was less numerous than these species but was more abundant than *P. peltatus* (Meigen) of which only two specimens were caught in 1953.

Melanostoma species were few in 1952 but about equally abundant in the

TABLE IV

Weekly totals of 11 species of Syrphidae in traps 1, 2 and 4 at Rothamsted Lodge garden in 1951, 1952 and 1953. (Maxima in heavy type).

		April			May				June				July					August				September				October					
	Week ending:	16	23	30	7	14	21	28	4	11	18	25	2	9	16	23	30	6	13	20	27	3	10	17	24	1	8	15	22	29	
	Week No:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	
	Trap No.																														Totals
M. mellinum	1	—	—	—	—	—	—	0	1	2	0	0	0	2	0	3	17	26	22	30	11	6	3	—	—	—	—	—	—	—	123
	2	0	0	0	3	3	1	1	0	1	0	1	1	4	8	14	**14**	7	1	0	0	1	0	0	—	—	—	—	—	—	60
	4	0	0	0	0	0	0	0	0	0	0	0	0	1	1	6	22	8	23	32	17	12	6	2	1	0	2	0	0	0	133
P. albimanus	1	—	—	—	—	—	—	1	3	5	4	1	1	3	1	5	6	17	11	10	9	9	8	—	—	—	—	—	—	—	94
	2	0	0	0	0	8	10	5	1	0	0	0	8	11	7	22	8	5	1	1	2	1	1	0	—	—	—	—	—	—	91
	4	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	8	27	13	7	0	3	0	0	0	0	0	0	61
P. clypeatus	1	—	—	—	—	—	—	0	0	0	0	0	1	4	1	2	12	27	19	24	7	0	1	—	—	—	—	—	—	—	98
	2	0	0	0	0	0	1	2	0	0	0	1	2	0	2	3	0	1	2	3	0	0	0	0	—	—	—	—	—	—	17
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4	2	**25**	11	8	0	0	0	0	0	0	0	0	0	51
S. balteatus	1	—	—	—	—	—	—	0	0	0	0	0	0	1	0	1	5	12	13	9	11	4	3	—	—	—	—	—	—	—	59
	2	0	0	0	0	0	0	0	0	0	1	0	0	0	3	**11**	5	2	0	3	3	0	0	0	—	—	—	—	—	—	28
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	5	6	16	21	12	8	1	1	2	0	0	1	0	0	75
S. vitripennis	1	—	—	—	—	—	—	0	0	0	1	1	0	2	2	5	3	19	11	6	3	6	4	—	—	—	—	—	—	—	63

	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4	6	3	2	1	0	1	0	1	0	0	19
M. scalare	1	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	0	2	4	3	1	2	2	—	—	—	—	—	—	—	14
	2	0	0	0	0	0	7	0	0	0	0	0	0	0	1	5	3	0	1	1	0	0	1	0	—	—	—	—	—	—	19
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	1	0	0	0	0	0	0	0	0	4
S. corollae	1	—	—	—	—	—	—	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	—	—	—	—	—	—	—	1
	2	0	0	0	0	0	0	0	0	1	0	1	0	0	2	3	0	0	0	1	0	0	0	0	—	—	—	—	—	—	8
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. peltatus	1	—	—	—	—	—	—	0	0	0	0	0	0	0	0	1	0	0	0	3	0	0	5	—	—	—	—	—	—	—	9
	2	0	0	0	0	0	0	0	0	2	2	1	6	3	0	0	0	0	1	1	1	0	0	0	—	—	—	—	—	—	17
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	2
S. pipiens	1	—	—	—	—	—	—	0	2	0	4	1	3	6	6	21	**28**	20	8	15	6	5	6	—	—	—	—	—	—	—	131
	2	0	0	0	0	3	14	40	0	14	8	14	**38**	21	23	20	11	10	6	3	6	2	0	0	—	—	—	—	—	—	233
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	5	3	**15**	10	6	14	11	1	3	0	2	0	0	0	74
Neoascia spp.	1	—	—	—	—	—	—	1	5	0	0	0	0	0	0	7	**10**	4	7	5	6	7	14	—	—	—	—	—	—	—	66
	2	0	0	6	2	21	10	3	0	0	1	0	5	2	3	**10**	4	2	2	1	1	1	0	0	—	—	—	—	—	—	74
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	**11**	1	7	2	9	3	0	1	1	1	0	0	0	0	39

other years. *M. mellinum* was the most common and the most abundant of all the aphidophagous Syrphidae caught, but it was comparatively scarce in 1952. *M. scalare* occurred infrequently and was trapped only during a brief period in each year.

Syrphus species were also few in 1952 but more species (11) were caught than in 1951 (7 species) and 1953 (5 species). Only two species of this syrphid were abundant: *S. balteatus* was usually the most numerous, although in 1951 its numbers were slightly exceeded by those of *S. vitripennis,* which was comparatively scarce in 1952. Other species of *Syrphus* were trapped infrequently, although in 1952, *S. corollae, S. auricollis* Meigen and *S. ribesii* were caught fairly often.

EXPERIMENT II. *Comparison of captures of Syrphidae in two traps situated in bean plots at different localities in 1952*

In 1952, two traps were operated in bean plots at two different localities at Rothamsted. One (trap 2) was at Rothamsted Lodge where there was a great variety of vegetation surrounding the beans; the other (trap 3) was one-quarter of a mile away in another plot, surrounding which were large fields of wheat, other beans and potatoes. The collections of Syrphidae in the two traps are compared for weeks 7—22 (22 May — 10 September).

TABLE V

Weekly totals of the more abundant species of Syrphidae in traps 2 and 3 (1952). (Maxima in heavy type).

		May	June				July					August				September		
Week ending:		28	4	11	18	25	2	9	16	23	30	6	13	20	27	3	10	
Week No:		7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
	Trap No.																	Totals
M. mellinum	2	1	0	9	0	1	1	4	8	**14**	14	7	1	0	0	1	0	61
	3	0	0	0	1	0	3	3	4	**12**	10	8	7	1	2	1	0	52
P. albimanus	2	5	1	0	0	0	8	11	7	22	8	5	1	1	2	1	1	73
	3	0	0	0	0	0	1	2	1	2	3	1	0	0	0	0	0	10
P. clypeatus	2	2	0	0	0	1	2	0	2	3	0	1	2	3	0	0	0	16
	3	0	0	0	0	2	6	1	2	7	8	4	0	1	2	0	0	33
S. balteatus	2	0	0	0	1	0	0	0	3	11	5	2	0	3	3	0	0	28
	3	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	3
S. corollae	2	0	0	1	0	1	0	0	2	3	0	0	0	1	0	0	0	8
	3	0	0	0	0	1	2	3	10	10	1	0	0	0	0	0	0	27
S. pipiens	2	**40**	0	14	8	14	38	21	23	20	11	10	6	3	6	2	0	216
	3	0	0	0	0	1	**7**	1	0	4	6	0	1	2	2	1	0	25

The two collections differed considerably (Table V). In trap 2, 531 Syrphidae (32 species) were caught whereas only 197 (17 species) occurred in trap 3; and, with the exception of *Platycheirus clypeatus* and *Syrphus corollae,* the numbers of each species in trap 2 exceeded those in trap 3.

The same five species of *Platycheirus* occurred in each trap but trap 2 caught more than twice as many individuals as trap 3. *P. albimanus* was by far the most abundant species of this genus in trap 2, whereas in trap 3, *P. clypeatus* was the dominant species and *P. albimanus* was scarce. *P. peltatus* and *P. scutatus* were fairly numerous in trap 2 but were scarcely represented in the other trap.

The two *Melanostoma* species were about equally abundant in each trap, and *M. mellinum* was the dominant aphidophagous species.

In trap 2, 58 specimens of 11 species of *Syrphus* were caught, whereas in trap 3 there were 36 specimens of only 6 species. In trap 2, *S. balteatus* was the most abundant species of this genus and there were few *S. corollae*; in contrast, in trap 3, *S. balteatus* was scarce and *S. corollae*, the dominant species, was abundant; as it happened, the total numbers of each of the dominant species were almost identical. *S. vitripennis* occurred in trap 2 but not in trap 3 (Table V).

There were also many more *Syritta pipiens* and *Neoascia* spp. in trap 2.

It seems that the syrphid fauna of the two localities differed. In trap 2, where the surrounding vegetation was more varied, the diversity of the captured Syrphidae was greater and the insects were much more abundant. The composition of the catches also varied with the locality. *P. albimanus* and *S. balteatus* were abundant in trap 2 but were rare in trap 3; and in trap 3, *P. clypeatus* and *S. corollae* were the most abundant aphidophagous species after *M. mellinum*. The differences between the collections in these two traps are emphasised by the contrast in their indices of diversity which differ significantly from each other (Table II).

EXPERIMENT III. *Comparison of captures of Syrphidae in two different types of vegetation.*

In 1953, two traps were sited near each other in the garden at Rothamsted Lodge. One (trap 4) was in the middle of a small plot of beans; the other (trap 5) was in the middle of a small plot of mixed weeds (p. 112). The traps ran concurrently and the collections of Syrphidae are compared for weeks 13—27 (3 July — 15 October).

In such a small area, two identical traps would be expected to collect equal numbers of randomly flying insects, although the type of vegetation surrounding the traps might also influence the composition of the catches. But the catches of some species in each trap were so similar that it seems likely that they were not greatly affected by the type of vegetation close to the traps, and that the traps were sampling equally from the syrphid populations of the garden. But for other species, the two sets of catches differed. Table VI shows the catches of the most important species.

In trap 5 (in the weeds), 503 Syrphidae (22 species) were caught and 558 (18 species) occurred in trap 4 (in the beans); 16 species occurred in both traps; the notable absentees from trap 4 were *S. corollae* and *Eumerus* sp. of which trap 5 caught 5 specimens of each. Captures of the remaining species were so few that no comment is possible.

The two sets of captures of the species *Platycheirus albimanus, Syrphus*

TABLE VI

Weekly totals of the more abundant species of Syrphidae in traps 4 and 5 (1953).
(Maxima in heavy type).

		July				August				September				October			
Week ending :		9	16	23	30	6	13	20	27	3	10	17	24	1	8	15	
Week No :		13	14	16	16	17	18	19	20	21	22	23	24	26	26	27	
	Trap No.																Tota
P. albimanus	4	1	1	0	0	1	8	**27**	13	7	0	3	0	0	0	0	
	5	0	0	0	0	0	5	**24**	13	5	3	1	2	0	0	0	
S. balteatus	4	0	1	1	5	6	16	**21**	12	8	1	1	2	0	0	1	
	5	0	0	0	1	4	**26**	22	8	5	4	0	0	1	0	0	
S. vitripennis	4	0	0	0	0	1	9	**10**	1	2	0	1	0	0	0	0	
	5	0	0	0	0	1	6	**10**	3	1	2	1	3	0	0	0	
P. scutatus	4	0	0	0	0	0	1	4	**6**	3	2	1	0	1	0	1	
	5	0	0	0	1	2	4	**11**	10	8	0	0	1	0	0	0	
M. mellinum	4	1	1	6	22	8	23	**32**	17	12	6	2	1	0	2	0	1
	5	0	0	4	7	8	35	**51**	31	18	6	2	1	1	0	0	1
P. clypeatus	4	0	0	1	4	2	**25**	11	8	0	0	0	0	0	0	0	
	5	0	0	0	0	0	**13**	12	5	11	3	0	0	0	0	0	
S. pipiens	4	0	0	4	5	3	**15**	10	6	**14**	11	1	3	0	2	0	
	5	0	0	2	3	0	5	9	15	15	**16**	4	2	1	0	0	
Neoascia spp.	4	0	0	3	**11**	1	7	2	9	3	0	1	1	1	0	0	
	5	0	0	1	0	2	2	3	5	**7**	4	0	0	0	0	0	

balteatus and *S. vitripennis,* closely resembled each other, separately, in several respects: the total numbers of each species caught, the sizes of the maxima, the times when the maxima were recorded, and in the time-distributions of each of these species.

In contrast, the captures of *P. scutatus* and *Melanostoma mellinum* resembled each other, separately, in their time-distributions and when the maxima were recorded; but *P. scutatus* was twice as numerous in trap 5, and *M. mellinum* was more abundant in trap 5. *P. peltatus* and *P. manicatus* were scarce in both traps.

The captures of *P. clypeatus* (in each trap, the second most abundant species of this genus) were, however, dissimilar in all respects.

The totals of *Syrphus* spp. in each trap were very similar (trap 4, 105; trap 5, 111). As already noted, the captures of *S. balteatus,* and of *S. vitripennis,* were closely similar in all respects; the totals of *S. auricollis* were almost equal in each trap, as were those of *S. ribesii.* Trap 5 caught five specimens of *S. corollae* which was entirely absent from trap 4.

Of the non-aphidophagous species, *Syritta pipiens* was equally abundant in each trap. It occurred at the same times, and maximum numbers were recorded approximately together. In trap 4, *Neoascia* spp. were more abundant and maximum numbers occurred earlier than in trap 5. *Cheilosia* spp. occurred in both collections but nearly three times as many were caught in trap 4 as in trap 5.

The two traps seemed to sample more or less equally from the populations of each of the species present in the garden. Support for this comes from the fact that the indices of diversity of the two collections are almost identical (Table II), which means that the differences in size and composition of the collections can be attributed mainly to chance. *P. albimanus, S. balteatus, S. vitripennis* and *S. pipiens* were apparently distributed fairly evenly over both lots of vegetation during the period of trapping. *M. mellinum* and *P. scutatus* were, however, somewhat more numerous around the weeds, and *P. clypeatus* seemed to be concentrated at first around the beans, later, around the mixed vegetation.

It is interesting to compare these conclusions with those of SOUTHWOOD (*personal communication*) who analyzed the catches of Heteroptera in these same two traps. He found twice as many individual Heteroptera in trap 5 as in trap 4, and attributed this to the fact that the plants around trap 5 were host plants of many of the species caught in that trap. However, the Heteroptera in trap 4 were more diverse. Southwood concluded that the catches of Heteroptera in suction traps and light traps is much influenced by the proximity of the insects' host plants to the traps.

DISCUSSION

Only six of the 32 species of aphidophagous syrphids caught in the traps could be called abundant, and of these, *Melanostoma mellinum* and *Platycheirus albimanus* were more numerous than other species. *Syritta pipiens* was the most abundant non-aphidophagous form.

The numbers of individual species recorded varied from year to year, with the time of year and from place to place. In 1951 and 1953, aphidophagous syrphids were more abundant than in 1952, a fact which is attributed to the differences in weather; but in 1952, the species commoner in other years were less numerous and scarce species were more numerous. Most species showed a small peak of numbers in late spring and a main peak in late summer, which may represent two broods. *Melanostoma scalare* showed a peak in mid-summer, and this species may normally have only one brood in the year, just as *Syrphus eligans* (Harris) (= *bifasciatus* (Fabricius)) has in Switzerland (SCHNEIDER, 1948). The abundance of a particular species also varied with the locality where the traps were sited, and appeared to be associated with the variety of the vegetation of the locality.

Weather, vegetation, aphid abundance, parasitoids and disease are but a few of the factors which can affect the abundance of Syrphidae.

WILLIAMS (1940) showed that the number of insects caught in a trap in one day depends on the activity of the insects and on the total population from which he day's sample is drawn. Changes in activity can best be estimated from differences between catches on successive days. The daily catches of Syrphidae in the suction traps were so small that effects of weather on changes in activity could not be estimated. The daily catches were totalled for each week and the differences between the weekly totals can be attributed mainly to changes in population size. Differences in size of catches from year to year depend even more on population size, as Williams pointed out.

No association could be found between the size of the catch of individual species, or of the totals of Syrphidae, and changes in weather, either before or at the time of capture. Most were caught when the annual temperatures were high; catches at other times of the year were too small to allow any conclusions to be drawn.

Nevertheless, weather seems to affect the abundance of Syrphidae in several ways. The results described here, and those obtained by general collecting and observation in other years, suggest that aphidophagous Syrphidae are more abundant in cool wet summers than in warm dry summers. The contrast in the abundance of individual species and the times of their occurrence shown by the results of trapping in 1952 and 1953 is particularly striking and, no doubt, is linked with the differences in the weather of the two years. Winter conditions may also affect the survival of overwintering Syrphidae which pass the winter as mature larvae, but winters in England are not usually severe, and the mortality of hibernating Syrphidae from adverse weather is unlikely to be high.

The food supplies of the adults and larvae seem to be important factors affecting abundance. The adults feed on the pollen and nectar of flowering plants, and I have also seen them feeding on the honeydew of aphids. According to SCHNEIDER (1948), the times of appearance and abundance of Syrphidae are associated with the times of flowering of various plants whose pollen and nectar the insects need to mature their ovaries. In those localities where flowering plants are abundant and provide a succession of mature flowers (as, for example, at Rothamsted Lodge garden), syrphids would be expected to be more plentiful and more diverse than in fields covered with wide stretches of more or less uniform annual crops (like those, for example, surrounding trap 3). Such fields provide neither the variety of flowers necessary for a continuous supply of food for adult Syrphidae, nor a permanent habitat for the shelter and overwintering of adults and larvae. But the results of Experiment III indicated that the kind of vegetation immediately around a trap did not seem greatly to affect the size and composition of the catch of Syrphidae, as it did affect the catch of Heteroptera (SOUTHWOOD, *in litt.*).

The abundance of Syrphidae must be strongly influenced by the abundance of aphids, which the adults have to find to lay their eggs, and which the larvae need as food. The adult syrphid is very selective of oviposition sites and lays its eggs close to aphid colonies, so that the emerging larvae are at once close to their food. Syrphid eggs rarely occur, for example, on bean plants uninfested with aphids, and there is a close correlation between the numbers of syrphid eggs and larvae and the numbers of aphids of individual bean plants (BANKS, 1952). Therefore, the flies will lay few eggs when aphids are scarce and few larvae will be produced, thus affecting the size of syrphid populations later on. The captures of adult Syrphidae in the suction traps in relation to the aphid infestations of bean plots will form the subject of another paper.

Aphidophagous syrphid larvae are attacked by several parasitoids, notably the ichneumonid, *Diplazon* spp., and the chalcid, *Bothriothorax* spp., both of which I have found in larvae at Rothamsted. I have also found syrphid larvae diseased from an unknown cause on bean plants. There is no information on the number of syrphid larvae that are killed.

My results provide preliminary information on the abundance of the commonest species of Syrphidae and indicate the extent of changes from year to year. But the method of assessing changes in the populations by trapping has its limitations, and it is desirable that information should be got by other methods of sampling.

I am grateful to Mr R. L. Coe of the British Museum (Natural History) for his help with the indentification of the more difficult species of Syrphidae. I wish to thank Mr L. R. Taylor for his comments on an early draft of this paper, and Dr T. R. E. Southwood for allowing me to read, and to quote from, his unpublished paper on Heteroptera.

ZUSAMMENFASSUNG

VERSUCHE ZUR ERMITTLUNG DER HÄUFIGKEIT VON SCHWEBFLIEGEN (DIPTERA, SYRPHIDAE) MIT SAUGFALLEN, UNTER BESONDERER BERÜCKSICHTIGUNG APHIDIVORER ARTEN

Ein vorläufiges Bild über die Häufigkeit einiger gewöhnlicher Syrphidenarten ließ sich aus Fängen von 5 Saugfallen gewinnen, die während der Sommer 1951, 1952 und 1953 in Rothamsted arbeiteten. Unter den 8 häufigen Arten befanden sich 6 aphidivore.

Die Individuenzahl der einzelnen untersuchten Arten schwankte von Jahr zu Jahr, im Jahresablauf sowie von Ort zu Ort.

Obwohl die Fänge nicht zu den unmittelbaren Wetterbedingungen in Beziehung gesetzt werden können, so könnten die großen Unterschiede zwischen ihnen doch mit den Unterschieden im allgemeinen Witterungsverlauf der 3 Jahre verbunden sein. Im Frühling gab es nur wenige Schwebfliegen, obwohl die meisten Arten dann einen kleinen Häufigkeitsgipfel aufwiesen; die größten Fangzahlen wurden bei fast allen Arten im Spätsommer (August) festgestellt.

An zwei verschiedenen Orten bestanden auffällige Unterschiede in der Häufigkeit der einzelnen Arten und in der Zusammensetzung der Fänge. Wo die Pflanzenbestände wechselten, waren die Syrphiden verschiedenartiger und häufiger als dort, wo die Vegetation gleichförmig war. Einige Arten erschienen an einem Ort, aber nicht an dem anderen. Die Größe und Zusammensetzung des Fanges wurden von der Art der Vegetation in der unmittelbaren Umgebung der Falle nicht merklich beeinflußt.

Einige der vielen Faktoren, welche die Häufigkeit der Syrphiden beeinflussen, werden besprochen.

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DIFFERENTIAL HOST RELATIONS OF THE SEXES IN PARASITIC HYMENOPTERA[1])

BY

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The divergent ontogeny of the male, a unique phenomenon characterizing certain genera of the Aphelinidae, has its physiological bases in the environmental control of sex permitted by the haploid origin of the male and the diploid origin of the female and in the efficiency of the chalcidoid type of spermatheca which, during the process of oviposition, brings the environmentally activated sperm into contact with the egg. This divergence is correlated with the sexual dimorphism and the sexual ditrophism of the early developmental stages. It is psychologically manifested in a number of species by a marked difference in the host relations of the aphelinid female before and after mating. The climax of differential host relations within a species is the production of the female in a coccid host and the production of the male in a lepidopterous host. The aberrant development of the male tends to increase the capacity of the species to survive at low host densities.

The known instances of differential host relations of male and female parasites in the Hymenoptera occur in certain species of the Aphelinidae. These species being relatively few are considered to be aberrant, the host relations of the hymenopterous male in most if not all other species being the same as those of the female rather than markedly different.

This aberrant differentiation between the sexes is expressed only by the ontogeny of the male. There is no differentiation in the form or behavior of the male adult. Aberrant differentiation is correlated in the majority of instances with differential deposition by the parent female of haploid and diploid eggs.

The aphelinids characterized by this peculiar phenomenon (FLANDERS, 1953b) reproduce as endoparasites of diaspine scale insects, mealybugs, and aleyrodids.

The aberrant male ontogenies of the several species are characterized by sex-limited hyperparasitism, ectoparasitism, and the kind of host insect attacked. As a consequence this reproductive phenomenon involves (1) a sexual ditrophism extending to two orders of the Insecta, namely to the Homoptera, and to either the Hymenoptera or the Lepidoptera; (2) a sexual dimorphism of the egg and early larval instars; and (3) the unisexual encapsulation of the egg and larva by the blood cells of the host.

Aberrant ontogenies of the aphelinid male were first recorded by TIMBERLAKE (1913) and FLANDERS (1925). TIMBERLAKE found that when the egg of *Coccophagus lycimnia* (Wlkr.) was deposited externally on a full-grown larva or pupa of a parasitic chalcidoid instead of in the body fluids of *Coccus hesperidum* (L.), its sex was changed unaccountably from female to male, a change which took place in the field as well as in the laboratory. FLANDERS observed a larva of *Prospaltella* moving nematode-fashion in the egg of the codling moth, *Carpocapsa pomonella* (L.). This species of *Prospaltella* presumably reproduced only as a parasite of scale insects, yet three per cent of 1,000 codling moth eggs yielded male adults of *Prospaltella.* The fact that only adult males of *Prospaltella* emerged from the moth eggs was of no particular interest at the time. Both cases of aberrant ontogeny were considered accidents of nature. The idea that in these species sex differentiation in host relations was not merely an incidental occurrence but was essential for their existence, was too farfetched to be even considered.

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Clues which could have led to the recognition of such a cryptic host-parasite relation were present in studies such as those of TAYLOR (1935) on the development of hyperparasitic males in *Casca parvipennis* Gahan and *Physcus intermedia* Gahan. Although TAYLOR'S rearing of *P. intermedia* showed that only the female developed as a primary parasite of *Chrysomphalus aonidum* (L.) and that the male was a secondary parasite of this scale, specifically and "deliberately" parasitizing its own species, he could suggest only that such male production was an effect of larval nutrition, the change from female production being effected either by a limited quantity of food or by cannibalism. He concluded that parthenogenesis, being abnormal in this species, could scarcely have been the cause of the very remarkable method of male production.

The essential character of this phenomenon for species reproduction was first realized in 1935 (FLANDERS, 1936, 1937) during the routine work of receiving and propagating species of parasitic Hymenoptera imported into California by the Department of Biological Control of the University of California. Its discovery, as pointed out by Harry S. Smith at the dedication of the Biological Control Laboratory in Belleville, Canada, stimulated renewed interest in the biological control of homopterous insect pests.

This knowledge proved to be prerequisite for the successful propagation and establishment in California of *Physcus* sp. (near *flaviceps* Gir. & Dodd), an endoparasite of *Lepidosaphes beckii* (Newm.), and of *Coccophagus insidiator* (Dalm.), an endoparasite of *Gossyparia spuria* (Mod.). The fact that such knowledge has proven to be of practical as well as of academic interest (FLANDERS, 1952a, 1952b) is the justification for this presentation.

SCOPE OF ABERRANT ONTOGENY OF PARASITIC HYMENOPTERA AND ITS HISTORICAL ASPECTS

On the basis of aphelinid life-history studies during the past 20 years it now appears that sex differentiation in host relations characterizes the majority of species which are primary parasites of diaspine scale insects, mealybugs, and aleyrodids.

The species listed in Table I constitute about 80 per cent of all those whose biologies are known. The remaining 20 per cent consist of uniparental species such as *Casca silvestrii* Comp., *Encarsia formosa* Gahan, *Prospaltella berlesei*

(How.), and biparental species such as *Eretmocerus serius* Silv. (CLAUSEN, 1940). The writer is greatly indebted to his colleague Harold Compere for the identification of most of the species listed in this table.

TABLE I

Types of Male Development and Representative Species in the Aphelinidae Classified According to the Ovipositional Behavior of the Parent Female[1])

Ovipositional Behavior Unchanged by Mating

Not Hyperparasitic	Indirectly[2]) Hyperparasitic
Species depositing male eggs ectoparasitically[3])	Species depositing both male and female eggs endoparasitically in a healthy coccid host
s *Coccophagus* sp. (nr. *flavidus* Comp.)[4])	s *Coccophagus basalis* Comp.
s ,, *ochraceus* How.	m ,, *gurneyi* Comp.
s ,, *longifasciatus* Comp.[4])	m ,, *pseudococci* Comp.
	s ,, *trifasciatus* Comp.
	s ,, *insidiator* (Dalm.)
	d *Prospaltella perniciosi* Tower
	o ,, *smithi* Silv.

Ovipositional Behavior Changed by Mating

Not Hyperparasitic	Directly[2]) Hyperparasitic	
Species depositing male eggs endoparasitically	Species selecting parasitized hosts for deposition of male eggs	
	Ectoparasitically	Endoparasitically
o *Encarsia* sp.[5])	s *Aneristus ceroplastae* How.	d *Casca wanhsiensis* Comp.[6])
d *Prospaltella* sp.[5])	d *Coccophagoides* sp.	d ,, *parvipennis* Gahan
	s *Coccophagus caridei* (Brèthes)	s *Coccophagus baldassarii* Comp.
	s ,, *cowperi* Gir.	s ,, *capensis* Comp.
	s ,, *eleaphilus* Silv.	s ,, *nigritus* Comp.
	s ,, *hawaiiensis* Timb.	s ,, *rusti* Comp.
	s ,, *japonicus* Comp.	s ,, *scutellaris* Dalm.
	s ,, *lycimnia* (Wlkr.)	s ,, sp. nr. *yoshidae* Nak.
	s ,, *pulvinariae* Comp.	d *Physcus* sp. nr. *flaviceps* Gir. & Dodd
	s *Euxanthellus* sp.	d ,, ,, ,, *intermedia* Gahan
	d *Physcus testaceus* Masi	d *Prospaltella elongata* Doz.
	o *Prospaltella clypealis* Silv.	d *Prospaltella opulenta* Silv.

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[1]) The female develops as a primary endoparasite of homopterous insects. The kind of host for each parasitic species listed is indicated as follows: (o) = aleyrodids, (m) = mealybugs, (s) = soft scale insects, (d) = diaspine scale insects. The male of most species develops as a hyperparasite of these hosts.

[2]) As defined by SMITH (1916).

[3]) The depletion of the spermathecal gland causes the female to oviposit ectoparasitically.

[4]) Classification based on unpublished studies by T. W. FISHER, Assistant Entomologist, University of California.

[5]) Endoparasitic in eggs of Lepidoptera.

[6]) Embryonic male probably a primary parasite of the larval female, the larval male a primary parasite of the coccid host.

In each of the species characterized by sex differentiation in host relations the differentiation is manifested by the behavior of the male either as an embryo or as a first-instar larva, or both, the adult male not being aberrant in any way. In such species, as in most Hymenoptera, the parental female must not only produce a new individual but also place that individual in a situation where it is likely to continue its development. This placement is accomplished by either of two methods: one (common to a few aphelinids) is by the deposition of a haploid egg and of a diploid egg in the same host individual; the other, a more common method, is by the deposition of a diploid egg in an immature homopterous individual and of a haploid egg in or on the larva or pupa of a hymenopterous parasite or in a lepidopterous egg.

The haploid egg may be placed (1) in the body fluids of a secondary (coccid) host, as with *Coccophagus gurneyi* Comp.; (2) in the body fluids of a parasitic host, as with *C. capensis* Comp.; (3) in the yolk of a lepidopterous egg, as with certain species of *Prospaltella* and *Encarsia*; (4) on the air-exposed outer body wall of an immature endoparasite, as with *C. lycimnia;* (5) on the air-exposed inner body wall of a mummified coccid, as with *Physcus testaceus* Masi; and (6) on the ventral outer body wall of a healthy coccid, as with *C. ochraceus* How. The host parasite differentiation characterizing the first placement method is established by the cannibalistic action of the larva derived from the haploid egg; that characterizing methods (2), (3), (4) and (5) is established by the differential oviposition of the parent female, the kind of host attacked before mating differing from that attacked after mating.

The fact that hymenopteran species which are biparental in reproduction can be classified according to the effect of mating on the oviposition responses of the female has been known since 1909, when Harry S. Smith (HOWARD & FISKE, 1911) found that mated females of *Melittobia* deposited significantly more eggs than did unmated females. Subsequently, SCHULZE (1926) observed a contrary situation in *Trichogramma,* the unmated female depositing more eggs than did the mated female. The psychological effect of mating on the behavior of the female, however, attained an extraordinary degree of development in many species of the Aphelinidae, host preferences before mating being markedly different from those exhibited after mating.

Unmated females of *Coccophagus gurneyi* oviposit more or less indiscriminately in all species of *Pseudococcus.* Mated females, however, may show marked discrimination in their selection of hosts (BESS, 1939). The mated female, when presented synchronously with two host species in which her progeny can develop with equal facility, such as *P. gahani* Green and *P. adonidum* (L.), deposits most of her eggs in *P. gahani.*

When the essential nature of sex differentiation in host relations was unknown, the propagation and subsequent establishment of certain aphelinid species was greatly handicapped, and in certain instances prevented. In their attempt to introduce *Prospaltella divergens* Silv. from Malaya into Cuba, CLAUSEN & BERRY (1932) experienced difficulties that must have been due to the deviate development of the male. A shipment of *P. divergens* made in Wardian cases stocked with *Aleurocanthus woglumi* Ashby produced several generations enroute and, upon arrival, yielded approximately one thousand adult females. As no males appeared, it was assumed that the species reproduced unisexually. A few of the adult females were liberated in the field, others were liberated on infested plants in an insectary, and still others were liberated on infested plants enclosed in cellophane bags. Not a single individual was reared from this number, and the stock was consequently lost. No satisfactory explanation of this loss could be found at the time, and it remained a mystery until the discovery of sex differentiation in host relations. It was then recalled that the shipment inadvertently contained a small number of *Eretmocerus serius*, which may have been the host of a sufficient number of males to maintain the stock while enroute from Malaya (CLAUSEN, 1940).

The production of males in certain species of the genus *Casca* parasitic in diaspine scale insects still present a particularly baffling problem (FLANDERS *et al.*, 1958). In most of the known species of this genus the production of males requires the presence of the immature female, but the exact relation between the immature sexes is unknown. Mated females of *C. chinensis* How., a specific parasite of the California red scale, *Aonidiella aurantii* (Mask.), and those of an undescribed species of *Casca*, a specific parasite of the yellow scale, *A. citrina* (Coq.), produce female offspring only. The eggs of unmated females, when deposited in the body fluids of the diaspine scale, remain in diapause. Such eggs, if they had heen fertilized, would have developed into female adults.

The laboratory propagation of a geographical race of *Casca wanhsiensis* Comp., a race similar in habit to *C. chinensis* and also parasitic in California red scale, was achieved without the knowledge that the unmated female could not produce progeny in hosts which had not been exposed to attack by the mated female. In 1948 a culture of *C. wanhsiensis* was initiated in California with 220 males and females collected in Szechuan Province, China, during a 5-day period beginning October 17. This species reproduced from the imported material so readily that it was considered to lack sex differentiation in its host relations. The releases of *C. wanhsiensis* were handled according to this misconception. Many thousands were colonized but not established, presumably because the sequence of the releases and the host densities upon which the releases were made were unsuitable for the establishment of such parasites.

Several additional species, however, have been propagated in the laboratory, and four species have been established in new environments without a knowledge of the males' aberrant life histories (CENDANA, 1937; SAWADA, IDEDA & TANAKA, 1932: SMITH & COMPERE, 1926, 1928; COMPERE & SMITH, 1932).

The first of such species were established in the vicinity of Riverside, California. A species from Africa, *Coccophagus capensis*, whose males develop parasitically in their own females, was established on infestations of black scale, *Saissetia oleae* (Bern.), by the release of 78 presumably mated adults at intervals during the fall and winter of 1924-25. The timing of these releases was not premeditated, but the reproductive conditions thus accidentally created were suitable for the continued production of both sexes. In other localities where much larger numbers were released during the preceding decade, establishment did not occur (SMITH & COMPERE, 1926).

About the same time and in the same place another African parasite, *Coccophagus trifasciatus* Comp., whose males rarely if ever parasitize their own females but must parasitize females of related species such as *Coccophagus lycimnia* in *Coccus hesperidum,* became established as the result of releasing 168 females in the same accidental manner.

COMPERE (1925) reported that propagation of *Coccophagus trifasciatus* in the laboratory failed because of the lack of males at the proper time. One lot of unmated females reproduced freely but their progeny were all of the male sex. These males undoubtedly develop on the full-fed larvae of *C. anthracinus* Comp. which were being reared in large numbers (SMITH & COMPERE, 1928). In 1927 an Australian species, *C. gurneyi,* parasitic in the mealybugs *Planococcus citri* (Risso) and *Pseudococcus gahani,* was established in California (COMPERE & SMITH, 1932) without the knowledge that its male developed only at the expense of larval chalcidoids that inhabit mealybugs.

Unmated females of *Coccophagus gurneyi* and *C. trifasciatus,* unlike those of *C. capensis,* oviposit as readily in their homopteran hosts as do the mated females. This is apparently the case, also, with *Prospaltella smithi* Silv., according to Herbert D. Smith *(in litt.). P. smithi* was introduced by Silvestri into Japan in 1925 and effectively controlled the aleyrodid *Aleurocanthus spiniferus* Quaint. (SAWADA *et al.,* 1932). The males greatly outnumbered the females, presumably before the homopteran host became scarce and while there were numerous females of their own kind on which they could develop.

The obscure nature of aberrant male development is emphasized by the fact that TIMBERLAKE (1913), after an intensive study of the biology of *Coccophagus scutellaris* (Dalm.), concluded that it always develops as a primary parasite. Following the discovery of this phenomenon it was found that the male-producing egg of *C. scutellaris* is deposited only in a newly hatched female larva and that this female must complete its larval development in order to be adequate as food for the male.

Establishment of a species whose females are characterized by differential oviposition, its hosts before mating differing in kind from those after mating, is most likely to be effected if either of two conditions prevails: (1) When the releases consist only of unmated females, the immature instars of other parasite chalcidoids suitable for the development of the male of the released species must be available at the release point, and a follow-up release must be made when the male becomes adult. (2) When the releases consist of mated females, such releases must be made in sequences which will ensure the presence of unmated females when the immature female instars of the same species are available as hosts of the male.

Establishment of such species in a new habitat may fail, however, in spite of their releases being handled according to the needs of their male development (FLANDERS, 1942c). This was the case with *Physcus testaceus,* a parasite of *Lepidosaphes ulmi* (L.), and with *Coccophagus caridei* (Brèthes), a parasite of lecaniine scale insects. *C. caridei* was colonized in large numbers in southern California, where it reproduced for only one generation (FLANDERS, 1936).

From the standpoint of laboratory propagation, sex differentiation in host relations is least complicated with a species such as *Coccophagus ochraceus,* both sexes of which develop as primary parasites of the same homopterous host. Although *C. ochraceus*, as a species, reproduces endoparasitically, its male develops ectoparasitically. CENDANA (1937) in 1934 readily propagated *C. ochraceus* in the laboratory. He observed the full-fed ectoparasitic larvae entering the host's body in order to pupate and thought that such larvae represented both male and female. At that time there was no reason for thinking otherwise. Not until six years had elapsed after sex differentiation was found to be obligatory in other species of *Coccophagus* were tests made to ascertain the occurrence of absence of sex differentiation in *C. ochraceus* (FLANDERS, 1942a).

Divergent development of the sexes attains its extreme and most apparent aspect when the female develops as a parasite of a homopterous insect and the male as a parasite of lepidopterous eggs. An interesting situation recently occurred in Peru (BEINGOLEA, 1958). As reported to the writer *(in litt.),* two species of *Encarsia,* endoparasites of two cotton-infesting aleyrodids are involved. The males of each *Encarsia* species develop endoparasitically in the eggs of a cotton-infesting leaf worm *Anomis texana* Riley. Prior to its taxonomic differentiation into two species the entomologists of the Estación Experimental Agricola de la Molina were particularly interested in *Encarsia* because for some time it had appeared to be one of the important parasites of *Anomis*. The minute size of *Encarsia* and its abundance in the field as an egg-parasite of *Anomis,* however, obscured the fact that continuous reproduction of the parasite on *Anomis* did not occur.

In 1958 Beingolea's attention was called to the fact that the reproduction of the known species of *Encarsia* was limited to aleyrodids as hosts and that the production from moth eggs was undoubtedly entirely male and similar in that respect to the life histories of *Prospaltella (Encarsia)* spp. which reproduce on diaspine scale insects but also produce males on lepidopterous eggs, such as those of the walnut codling moth in California (FLANDERS, 1924, 1925) and of the southwestern corn borer, *Diatraea grandiosella* Dyar, and the corn earworm, *Heliothis armigera* (Hbn.), in the cornfields of northeastern New Mexico (DAVIS, HORTON, GABLE & BLANCHARD, 1933).

V. L. Wildermuth of the U.S. Department of Agriculture Bureau of Entomology and Plant Quarantine (in a letter to the writer dated September 19, 1935) stated that only the male of *Prospaltella* was reared from the moth eggs in New Mexico. The extraordinary fact that the male of certain aphelinid species which reproduce only as parasites of diaspine scale insects and aleyrodids develops as a primary parasite of lepidopterous eggs rather than as a secondary parasite of such eggs, was first definitely demonstrated in 1958 by Oscar Beingolea.

The *Prospaltella* which in 1924 and 1925 was observed in California to parasitize the codling moth egg on walnuts was as a rule solitary in its development. However, two small, very active larvae were observed in the yolk of one moth egg. Four days later one had considerably outgrown the other. At the end of seven days only one remained. The *Prospaltella* pupa, when newly formed, is light yellow and lies within the host egg shell surrounded with pellets of brown excreta. The egg shell immediately above the newly formed pupa becomes fuscous losing its transparency. The pupal period is from two to four weeks. Curiously one moth egg, which was obviously parasitized, recovered and 26 days after its discovery yielded a codling moth larva. This occurred at temperatures when the incubation period of the codling moth egg averaged 12-1/2 days (FLANDERS 1925).

The versatility of the male in the genus *Prospaltella* is manifested by its hyperparasitization of mealybugs and of diaspine scale insects (FLANDERS, 1951; FLANDERS, GRESSITT & FISHER, 1958).

It is noteworthy that a supposedly similar phenomenon was observed in 1915 by WILLIAMS (1916) in the aphelinid *Ablerus (Azotus) clisiocampae* (Ashm.). In this case, however, both sexes were reared from eggs of the lepidopterous host, the eastern tent caterpillar, *Malacosoma americanum* (F.), as well as from the coccid hosts *Aspidiotus* sp. and *Chionaspis* sp. Williams noted that four other species of endoparasites were associated with *Ablerus* and that the latter "oddly enough" was always the last to make its appearance. The significance of this emergence sequence was not appreciated until CLAUSEN & BERRY (1932) observed a species of *Ablerus* to reproduce hyperparasitically, the female being secondary in its development rather than primary on these homopterous and lepidopterous hosts. So far as is known, *Ablerus* spp. show no sex differentiation in host relations developing only as endoparasites of other hymenopterous parasites. This complete hyperparasitism represents one extreme in the various types of aphelinid development, the other being represented by *Eretmocerus serius*, both sexes of which develop only as primary parasites of aleyrodids (CLAUSEN & BERRY, 1932).

The host relations of the subfamily Aphelinidae for many years appeared so indiscriminate that Harry S. Smith, in a letter to S. E. Flanders, dated April 27, 1925, stated: "The family to which Prospaltella and Ablerus belong, the Aphelinidae, seems to be rather primitive, with host-relationships which are not very finely established, so perhaps it should not be surprising to find such a parasite attacking hosts differing so widely as scales and moth eggs, particularly in view of their superficial resemblance . . . it seems to me extremely doubtful if Prospaltella is normally a parasite of codling moth, or of any other lepidopterous, eggs."

L. O. Howard, a world authority on the Aphelinidae, in a letter to S. E. Flanders, dated November 4, 1924, questioned the rearing of *Prospaltella* from the eggs of the codling moth, stating that "the species of *Prospaltella*, so far as known...... were all parasitic upon Aleyrodidae with the possible exception of one or two from Diaspine scales." In a letter dated March 31, 1936, Howard commented that he had always been puzzled by the question of sex in this family, and that the males of many species had never been encountered.

HOWARD (1911), in tabulating the host relations of the genus *Coccophagus*, stated that the probabilities of development in species of this genus pointed towards invariable primary parasitism of coccids. This he would consider to be the case "...were it not for some of the extraordinary results of rearings carried on at the parasite laboratory at Melrose Highlands under the direction of Mr. W. F. Fiske and of others at the boll-weevil laboratory at Dallas under the direction of Mr. W. D. Hunter."

These rearings were presumably similar to those of TIMBERLAKE (1913), who observed that *Coccophagus lycimnia*, although a primary endoparasite of *Coccus hesperidum* and regularly thelyotokous, producing females generation after generation without the intervention of the male, also produced males hyperparasitically, such males developing ectoparasitically on the full-grown larvae and pupae of *Microterys* sp. and *Metaphycus* sp. within *Coccus hesperidum*. This hyperparasitism appeared to be a radical change in larval habit of *Coccophagus lycimnia*. As a primary parasite it was regularly endoparasitic in *Coccus hesperidum*, but when hyperparasitic it was ectoparasitic on a primary inhabitant of the coccid. TIMBERLAKE noted that the male eggs were always placed upon the full-grown larva or the pupa of a primary parasite. These males did not appear to be essential for the reproduction of the species because when oviposition occurred in unparasitized *Coccus hesperidum* only female offspring were produced.

The economic importance of the aberrant ontogenies in the Aphelinidae results, in part, from the fact that the utilization of such species has been handicapped by the hyperparasitic oviposition of the unmated female.

In 1924, a species of *Euxanthellus* was sent to California from South Africa, where it was alleged to be an important parasite of the black scale. COMPERE (1931), in a study of the species in the Quarantine Insectary of the University of California Citrus Experiment Station, found that it would not oviposit in unparasitized hosts but would readily do so in parasitized ones. *Euxanthellus* placed its eggs on the larva of a primary parasite in essentially the same manner as *Coccophagus lycimnia*. Because of this hyperparasitism, however, the entire stock of *Euxanthellus* was destroyed, so that none was liberated. In 1953, a female of *Euxanthellus* was reared by the writer as a primary parasite on *Coccus hesperidum*, and the male was reared as an ectoparasite of the primary parasite *Metaphycus* sp.

SPECIES WHOSE UNMATED AND MATED FEMALES OVIPOSIT AS PRIMARY PARASITES

TOWER (1914) noted that when populations of the San Jose scale, *Aspidiotus perniciosus* Comst., were parasitized by *Prospaltella perniciosi* Tower, a solitary endoparasite, many host individuals contained unhatched eggs of *Prospaltella*. When these hosts also contained a nearly mature *Prospaltella* larva, a younger newly hatched larva entered the body of the older larva and fed upon its body fluids without, however, preventing it from maturing and voiding its meconium. The younger larva then developed rapidly and destroyed the older one. However, the fact that this younger larva was a male was not demonstrated until 20 years later when RICE (1937) found that in San Jose scale only one of two larvae hatched from eggs deposited at the same time completed its development and that it was male.

COMPERE & SMITH (1932) observed a similar phenomenon in the case of the mealybug parasite *Coccophagus gurneyi* and concluded that species of this genus in which larval development is solitary are capable under certain conditions of hyperparasitic development - that is, of development upon other individuals of its own species or upon other primary parasites of the host mealybug.

Whenever a previously parasitized host was attacked, it seemed evident (although not the fact) that the female oviposited on the primary larva in preference to the mealybug itself. The existence of such a habit, according to COMPERE & SMITH (1932), is of no economic significance since there is no selection by the parasite female of parasitized hosts in preference to unparasitized ones. These authors also noted that when *C. gurneyi* oviposited in the immune host *Planococcus citri* inhabited by the primary parasite *Leptomastidea abnormis* (Gir.), the latter, by consuming the host's body fluids and creating a dry condition, rendered itself vulnerable to the attack of the *C. gurneyi* larva. However, all of the observed larvae died and their sex remained unknown.

Further studies on *Coccophagus gurneyi* in 1933 by CENDANA (1937), a student of Smith's, revealed that none of the eggs deposited by the unmated female hatched, the first-instar larva remaining in the egg as long as it was immersed in the body fluids of the mealybug host. This "inhibited hatching" was then thought to be the result of the unmated condition of the parent female, and only when the unfertilized (male) egg was deposited by the mated female did it hatch and develop in the manner of the fertilized (female) egg.

The fact that the *Coccophagus gurneyi* female deposited both unfertilized and fertilized eggs in the body fluids of the same host, plus the fact that dissections of the host destroyed any larval parasite within it, so completely obscured the differential development of the sexes that its discovery depended on the study of a species whose females, before mating, selected a host of a kind different from that selected after mating.

DISCOVERY OF THE OBLIGATORY NATURE OF ABERRANT ONTOGENIES

The supposedly accidental hyperparasitism of aphelinids was found to be obligatory as a result of the collection of certain species of aphelinids in South America during 1934-35 by Harold Compere and the propagation of such species in California by the writer. One of the first of these aphelinid species collected was *Coccophagus basalis* Comp. In the locality where it was originally found it was the dominant parasite of the black scale, *Saissetia oleae.* During the last two weeks of September, 1934, South American black scale sent to Riverside yielded 108 adults of *C. basalis,* all of which were female. These females, when placed with suitable hosts (first- and second-instar black scales on *Cycas revoluta*) did not reproduce, although they readily oviposited in the scales. Dissection of the parasitized scales revealed the eggs to be unhatched, with a perfectly formed parasite larva in each. *Coccophagus basalis,* therefore, like *C. gurneyi*, appeared characterized by "inhibited hatching."

The cause of "inhibited hatching" was found to be the immersion of the egg in the body fluids of the coccid host, hatching being inhibited until such fluids are replaced by air. This replacement is effected by a companion parasitic larva either of the same or of different species. The discovery of this phenomenon was dependent on a combination of circumstances involving *Coccophagus caridei,* another South American parasite of black scale, associated there with *C. basalis* but one in which "inhibited hatching" does not occur (FLANDERS, 1939b).

Coccophagus caridei was reared from seven of Compere's Brazilian shipments and from two of his Argentine shipments. Although numerous live adults were reared from the Brazil material and their propagation was repeatedly attempted, no reproduction occurred until March 1935. Shipments were then obtained from Argentina which contained a quantity of the mealybug *Planococcus citri* parasitized by *Anagyrus pseudococci* (Gir.). The mealybugs were collected from host plants on which they were associated with infestations of black scale.

In the Argentine shipment, unlike the Brazil shipment, the males and females of *Coccophagus caridei* were equally numerous. All the females emerged from the black scale. The discovery of sex differentiation in host relations was dependent on the fact that the males for the most part emerged from the mealybug. A series of tests then demonstrated that the unmated female oviposited only as a hyperparasite of either lecaniine scale insects or mealybugs and that mated females oviposited only as primary parasites of *Saissetia* spp. and certain related soft scales. When released on citrus in California *Coccophagus caridei* reproduced in large numbers for a single generation as a parasite of *Coccus pseudomagnoliarum* (Kuw.). It did not become established, however.

The insectary propagation of *Coccophagus caridei* was contingent on the discovery that the oviposition habits of the female before and after mating are markedly different. The female before mating is a psychological secondary parasite, after mating a psychological primary parasite, a change correlated with sex differentiation in host relations.

With this knowledge it was possible by inductive reasoning to explain the "inhibited hatching" of the eggs of *Coccophagus basalis* and *C. gurneyi*. The fact that the male *C. caridei,* unlike the female developed in a dry environment established the possibility that the prerequisite for the hatching of the "inhibited eggs" deposited by the unmated females of *C. basalis* and *C. gurneyi* was a dry environment and that the larvae in such eggs possessed an open respiratory system. This possibility was tested by removing an inhibited egg from the host's body fluids and allowing it to dry. The only material then at hand for testing was *C. gurneyi.* This species was being used by BESS (1939) in the study of the phagocytic response of mealybugs to the presence of coccophagine eggs. At the writer's suggestion, Bess removed the "inhibited eggs" of *C. gurneyi* from the body fluids of the mealybug host. These eggs, upon being exposed to free air, hatched immediately. The larvae so produced were male planidia with open spiracles, ambulatory spines, and a body enclosed in heavily chitinized segmented plates. Each larva, upon hatching from the egg, enters the body of the primary inhabitant of the mealybug, develops endoparasitically until nearly full grown, then bores its way out and completes its development as an ectoparasite.

SEXUAL DIMORPHISM AND DIFFERENTIAL DEVELOPMENTAL BEHAVIOR

One of the peculiar characteristics of sex differentiation in host relations is the sexual dimorphism of the first- and second-instar larvae. The female, on the one hand, is always endoparasitic, and its first instar is typically chalcidoid, possessing a closed respiratory system. The male, on the other hand, exhibits marked structural differences, particularly in the first instar, thus differing not only from the female but from the males of other species of similar habit.

Endoparasitic first-instar males lack an open respiratory system and sometimes are teleaform with body surfaces densely or sparsely covered with hairs and sometimes bearing one or more pairs of ventral spines. Ectoparasitic first-instar males possess open spiracles, the abdominal segments encircled with ambulatory spines and/or several pairs of prolegs.

The endoparasitic larvae of *Coccophagus capensis* show sex differentiation in their responses to the nutritional status of their hosts, the female enclosing itself in a cellular trophamnion, the male not doing so (FLANDERS, 1942b). The endoparasitic first-instar male of *C. rusti* Comp., when derived from eggs deposited accidentally in the body fluids of a coccid rather than normally in the immature primary parasite of that coccid, will starve, being unable to penetrate the body wall of the primary parasite in order to obtain their proper nutriment (FLANDERS, 1939b).

Sex differentiation as a response on the part of the host is elicited by the eggs and newly hatched larvae of *Coccophagus gurneyi* when in species of mealybugs that are largely immune to destruction by *C. gurneyi* females, the female eggs and larvae then being readily encysted by the host's phagocytic blood cells. The male eggs with their unhatched larvae are not so encysted (BESS, 1939). A contrary situation apparently occurs when mated females of *C. caridei* oviposit in the body fluids of the soft scale *Coccus hesperidum*. In such a case some of the eggs are encapsulated by blood cells (FLANDERS, 1939). This response on the part of the host is presumably elicited by the unfertilized egg, only the fertilized egg being adapted to such an environment. The peculiar, cylindrical jet-black cysts, recently observed by the writer in the body fluids of healthy individual hosts in lecaniine scale populations parasitized by *Coccophagus basalis*, probably were formed around dead embryonic males these not having had companion parasites to provide them with conditions that would enable them to hatch.

In certain species of *Coccophagus* and *Euxanthellus* sexual dimorphism occurs in the deposited egg (FLANDERS, 1939a). This dimorphism is effected at the time of oviposition. For example, the unmated females of *C. lycimnia* and *C. caridei* oviposit only on the surface of cocooned primary larvae or pupae in scale insects or mealybugs. The deposition of such eggs has been observed in a mealybug which had been mummified by the primary parasite and had one end of the mummy removed to expose the primary parasite which serves as the host of the male. After an unmated female has drilled through the dried, hardened integument of the mummy and the enclosed host larva or pupa is within reach of the exploring ovipositor, an egg is deposited on such hosts. This occurs when the barbed tip of the ovipositor merely penetrates the soft integument of a host parasite. The egg issues from the ovipositor above the imbedded tip so that most of it lies on the host's surface. The anterior part of the egg, immediately before it is released from the ovipositor and while it is greatly attenuated, is thrust beneath the host's integument and twisted into a "knot". If this "knot" is allowed to remain in position for a few minutes it hardens and becomes melanized. The egg then cannot be removed without injury. This egg, if it had been deposited endoparasitically, would have been typically hymenopterous in shape.

EFFECT OF ABERRANT ONTOGENIES ON THE ECONOMY OF THE SPECIES

The survival value of sex differentiation in the host relations of the Aphelinidae is more or less obvious, the host-searching capacity of an adult parasite population being, in part, an effect of the relative abundance of females. In most if not all the populations of such aphelinids at low host densities, the females greatly outnumber the males, so much so, in fact, that with some species, such as *Coccophagus scutellaris,* competent observers have considered them to be entirely thelyotokous. As noted above the unmated female of *C. scutellaris* oviposits only in the newly hatched female larva of its own species. By means of its antennae it quickly recognizes the hosts containing such larvae. In aphelinid species of this type the male may have a marked attraction for the unmated female.

Field populations of parasitic Hymenoptera are, in general, predominantly female because sex is environmentally controlled (FLANDERS, 1942a). This high proportion of females is accentuated by sex differentiation in host relations. The production of males by unmated females is facilitated when immature females are relatively numerous. The consequently greater number of males increases the chances that the newly emerged female will be mated. This tendency to increase the proportion of immature females results from mated females having only female progeny, as does *Coccophagus scutellaris.*

In certain species, particularly those of the genera *Aspidiotiphagus* and *Prospaltella,* all adults are female, the species having become uniparental in reproduction by discarding the male. One noteworthy example is *P. berlesei,* an important parasite of *Pseudaulacaspis pentagona* (Targ.) (HOWARD, 1912). Another is *Prospaltella perniciosi* which, as a parasite of *Aspidiotus perniciosus,* is of particular interest, since in eastern North America it appears to be in the process of discarding the male. Under field conditions in that region males are quite common and readily mate with the females. The larval male undergoes most of its development parasitically in the mature larva of the female of its own species (TOWER, 1914). However, when propagated in the laboratory on *A. perniciosus* it reproduces generation after generation without males (FLANDERS, 1944). A geographical race of this *Prospaltella,* collected numerous times in East Asia and now established in California on California red scale, appears to be completely devoid of males, none having been observed either in the field or in the laboratory (FLANDERS, 1953a).

THE PHYSIOLOGICAL BASES OF ABERRANT ONTOGENY

The bases for the physiological, morphological, and psychological adaptation as manifested in sex differentiation in host relations may lie in the following hymenopterous characteristics:

(1) The quiescence of the sperm in the mated female and their activation by the secretions of the spermathecal gland.

(2) The facultative modification of the sex ratio permitted by the haploid origin of the male and the diploid origin of the female.

(3) The expression of preferential oviposition by environmentally induced activation of the spermathecal gland, provided that its secretionary capacity is adequate.

(4) The structure of the spermatheca in the chalcidoids being such that the environment may have an immediate influence on the sexuality of the egg at the moment of its deposition.

The correlated effects of these characteristics seem best exemplified in *Coccophagus ochraceus* in which sex differentiation in host relations is, in part at least, a function of the ovipositional behavior of the parental female.

The host preferences of this species are not changed by mating. In *Coccophagus ochraceus* the rate of oviposition indirectly causes a marked change in the oviposition responses of the female. This change apparently results from the fatigue or depletion of the spermathecal gland, this gland in all chalcidoids being relatively small. When a mated female is presented with an abundance of second-instar *Saissetia hemisphaerica* (Targ.), she will deposit a clutch of eggs in rapid succession - one per host. Most of the eggs thus deposited, however, are unfertilized and are deposited ectoparasitically beneath the host. Fertilized eggs, on the contrary, are deposited endoparasitically in the body fluids of the same host. The oviposition stance of the mated female when depositing a fertilized egg is markedly different from that taken when depositing an unfertilized egg. In the first case the female stands on the dorsum of the host and inserts its ovipositor vertically downward; in the second case the female stands beside the host and thrusts its ovipositor horizontally backward.

The change in oviposition stance is definitely correlated with the fertilization of the egg and therefore with the action of the spermathecal gland.

The mated female, when prevented from depositing an unfertilized egg by being brushed aside whenever it assumes the oviposition posture which is known to immediately precede the deposition of such an egg, will shortly thereafter deposit a fertilized egg. It is thus possible to force a female to deposit only fertilized eggs, eggs that otherwise would have been deposited unfertilized. Conversely, however, it is not possible to force a mated female to deposit only unfertilized eggs. If a female is brushed aside whenever she assumes the posture which immediately precedes the deposition of a fertilized egg, she will deposit none. Studies of the female anatomy show that the only mechanisms that would permit such regulation are the spermathecal gland and the correlated sensory system.

The secretion of the spermathecal gland, as in all Hymenoptera, is essential for the activation of the sperm. The stimulation of this gland in most parasitic Hymenoptera is host-induced. *Coccophagus ochraceus* will deposit an unfertilized egg under an individual host and a fertilized egg within that host. When the interval between ovipositions is long, the female deposits mostly fertilized eggs; when the interval is short, she deposits mostly unfertilized eggs. This indicates that when the oviposition rate is high there is a lag in the secretion of the gland as a result of either its fatigue or its depletion of secretions. This effect of rate of oviposition on egg fertilization is correlated with the structure of the spermatheca, particularly the relatively small spermathecal gland, and the relatively long chalcidoid sperm (FLANDERS, 1939a). When the micropyle of an egg moving along the oviduct is opposite the opening of the sperm duct, the head of an individual sperm may at that moment enter the micropyle of the egg, the tip of its tail still within the sperm capsule. The structure of the chalcidoid spermatheca and of the sperm permits the fertilization of the egg at the moment of its deposition to be an immediate response to environmental conditions.

It is apparent, therefore, that the depleted condition of the spermathecal gland as a result of rapid oviposition causes the female to change her oviposition response from that of an endoparasite to that of an ectoparasite. Normally, the gland does not remain depleted of secretion, and after a short interval the female again oviposits as an endoparasite.

The minimum time lapse between assumptions of the endoparasitic position by the mated female corresponds to the time needed for the spermathecal gland to become replenished and again become turgid. It is apparent that when the female stands on top of the host the gland is turgid so that the egg then deposited is necessarily fertilized. When the gland is flaccid the female stands beside the host, the egg then deposited being necessarily unfertilized. For such a phenomenon to be realized, it is, of course, essential that the spermathecal gland responds to host stimuli.

An unmated female, when first presented with a host, may deposit an unfertilized egg in the host's body fluids. Thereafter, however, all deposited eggs are placed externally, the spermathecal gland of the unmated female not responding to any host-derived stimuli. Unfertilized eggs, if deposited internally, do not hatch, for the larvae then would be in a lethal environment.

The lack of response of the spermathecal gland to oviposition stimuli and its consequent lack of turgidity may account for the predilection of the mated female of *Coccophagus caridei* for ovipositing in the mid-gut of the host when only the host's body fluids provide a medium suitable for the development of its female progeny (FLANDERS, 1939b). Such oviposition occurs most frequently in the immature rather than the mature stages of the host. When deposited in the host's mid-gut the egg disintegrates without embryonic development being completed.

The discovery that in *Coccophagus ochraceus* the male develops ectoparasitically and the female endoparasitically on the same host coccid makes questionable all earlier observations, such as that of SILVESTRI (1935) who considered that both sexes of a species of *Prospaltella,* which he found parasitizing *Chrysomphalus dictyospermi* (Morg.) in Argentina, were ectoparasitic in development. On the basis of this observation, apparently, he described this species as *P. ectophaga.*

RESUME AND CONCLUSIONS

Aphelinids characterized by an aberrant ontogeny of the male have been successfully used in biological control work demonstrating the capacity of such species to maintain the populations of their hosts at noneconomic densities. Such species include *Coccophagus gurneyi* and *Prospaltella smithi* whose ovipositional behavior is not changed by mating (COMPERE & SMITH, 1932;; PETERSON, 1955; SAWADA *et al.*, 1932). In these species males occasionally outnumber the females. SAWADA *et al.* (1932) noted that the males of *P. smithi* far outnumber the females. Such a sex ratio also occurs in laboratory cultures of *Casca wanhsiensis* (FLANDERS *et al.*, 1958).

Species in which ovipositional behavior is changed by mating also may be effective parasites. *Coccophagus rusti*, for example, is often the dominant parasite of *Saissetia oleae* infesting the oleander and the pepper tree, *Schinus molle*, in California.

It is apparent that the various attributes characterizing sex differentiation in host relations are in no way inimical to species reproduction. Species reproduction in less highly developed insects is insured by high fecundity. The oviposition sites of many parasitic Hymenoptera with synovigenic reproduction are so scarce and cryptic that the female is characterized by high searching capacity rather than by high fecundity. High searching capacity in the Hymenoptera results in part from the substitution of female for male progeny, a function which enables a species to exist at lower host densities than it would otherwise (FLANDERS, 1947).

The ability to produce a female in place of a male attains its highest development in the Aphelinidae. It appears essential in many biparental species of this subfamily for the female to oviposit more readily before mating than afterwards. In such species, as in *Coccophagus lycimnia*, the psychology of the female is markedly changed by mating, the female before mating ovipositing in the coccid host only hyperparasitically and after mating only as a primary parasite. Consequently the necessary male is produced only by the unmated female, the mated females producing only females. The female of such species before mating oviposits either in or on the immature forms of parasitic Hymenoptera or in the eggs of certain Lepidoptera and after mating only in the nymphs or adult females of Homoptera.

In the species characterized by sex differentiation in host relations the deviations of male development from that of the female and the remarkable variations exhibited by such development are ontogenetic aberrations which appear to be unrelated to the phylogeny of the group. The males of such species, when adult, show no significant deviations either in form or in habit. The fact that they are haploid in origin may indicate a concomitant decreased resistance or increased susceptibility of the germ plasm to modification by environmental influences. The ontogenetic aberrations of the aphelinid male show at least that haploidy permits an unusual freedom for gene combinations, combinations which persist.

ZUSAMMENFASSUNG

UNTERSCHIEDLICHE WIRTSVERHÄLTNISSE DER GESCHLECHTER BEI PARASITISCHEN HYMENOPTEREN

Nachdem 1935 die obligatorisch wirtsverschiedene Geschlechterentwicklung bei den parasitischen Hymenopteren entdeckt wurde, ist dieses Phänomen bei ca 80% der bekannten Apheliniden, die in Schildläusen und Mottenschildläusen schmarotzen, gefunden worden. Die Kenntnis dieses Phänomens ermöglichte die Züchtung und Ansiedlung von 2 Arten solcher Parasiten in Californien. Die wirtsverschiedene Geschlechterentwicklung besteht in der aberranten Entwicklungsgeschichte des Männchens sowie der geschlechtsgebundenen Ditrophie mit Dimorphismus der Larvenstadien. Bei einigen Arten ist sie gekoppelt mit psychologischen Unterschieden zwischen begatteten und unbegatteten Weibchen, die dann verschiedene Wirtspräferenzen zeigen. Diese bemerkenswerte Spaltung der Wirtspräferenzen steht wohl teilweise in Zusammenhang mit der Funktion der Spermatheca des Chalcidoiden-Typs, die äußerst empfindlich auf wirtseigene Stimuli anzusprechen pflegt. Es handelt sich um ein Wirts-Beziehungs-Phänomen, welches die Wirtsfindungsrate der Art zu erhöhen pflegt. Die ontogenetischen Abweichungen in der Entwicklung der Männchen dürften zur Phylogenie der Apheliniden in keiner Beziehung stehen.

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ABORTIVE FEEDING BEHAVIOUR IN A BLACK APHID OF THE *APHIS FABAE* GROUP

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A black *Aphis* sp. on *Tropaeolum* can be separated, morphologically, from *A. fabae* Scop. In controlled experiments this aphid, both winged and wingless forms, actively selects *Vicia faba* as a host plant, settles to feed and reproduce, but always dies prematurely. Offspring also soon die. The aphid is unable to take up plant sap from beans. The behaviour associated with host plant selection is not adjusted to its feeding requirements.

Behaviour in phytophagous insects usually appears to be highly adapted to finding and recognizing the most suitable host plants for the species. Migrant aphids often alight on unsuitable hosts when they disperse to new sites and these plants are rejected. The pattern of behaviour involved in host plant selection by both alatae and apterae of many of these aphids, in particular *Aphis fabae* Scop., has been well studied (e.g. KENNEDY & BOOTH 1951, JOHNSON 1958, MÜLLER 1958a).

It is interesting, therefore, to find a clear example of an aphid, closely allied to *A. fabae,* that will persist in settling down to feed and reproduce on a plant upon which neither it, nor its young, can live.

THE APHIDS USED

The aphid is a common member of the *fabae* complex of black *Aphis* spp. It has not previously been clearly separated from *A. fabae* Scop. although its existence has been suggested (e.g. PRICE-JONES unpublished). It probably has no name; here it will be called the Tropaeolum Aphid. EASTOP (unpublished), has separated it from the species commonly known as *A. fabae* Scop., *A. solanella* Theob. (= *A. euonymi* B. & J. ?), *A. podagrariae* B. & J. and *A. cognatella* Jones, by the length of the ultimate rostral segment. This is 0.14 - 0.20 mm in specimens 1.5 - 3.0 mm long compared with 0. 11 - 0.16 mm in *A. fabae* for example.

The species is commonly found on *Tropaeolum* (The Garden "Nasturtium") in the summer. How it overwinters is not known. No sexual forms have yet been found. Black aphids with a similarly long ultimate rostral segment have been found by Dr. Eastop on thistles. *A. fabae* also feeds on *Tropaeolum* and appears to be the only other aphid of this group which has been recorded to do so. *A. rumicis* is also reported as feeding on *Tropaeolum* but is easily separated from the other aphids. The Tropaeolum aphid has presumably been recorded in the past as *A. fabae* or *A. rumicis.* The behaviour of this aphid is compared with that of *Aphis fabae* Scop.

METHODS AND MATERIALS

A routine study of the life-history of individual *A. fabae* has been established. Fourth instar nymphs or newly moulted adults are put on separate, glasshouse grown, broad bean seedlings (*Vicia faba* L., Claudia Aquadulce) 2-3 in. high, sown four to a pot. The aphids can select a feeding site. They are moved only when the plants are changed every four or five days, or when they occasionally wander off their allotted plant. The nymphs they produce are counted daily and usually destroyed; the parents are sometimes disturbed when this is being done. The infested plants are kept in a ventilated cabinet at 20° C, 45% R. H. and have 16 hours reflected fluorescent light a day.

The alternative hosts used were *Tropaeolum majus* L. and *Forsythia intermedia* Zabel. Excised leaves and young shoots, respectively, were kept supplied with water in corked tubes which the aphids could walk off if they wished. Normally the leaves were changed every 2 or 3 days, but some of the *Tropaeolum* leaves were used for a longer period when the supply was interrupted.

The same cabinet was used for the measurement of rate of growth of nymphs but the nymphs were not kept on separate plants. A batch of young adults was placed on seedlings or leaves and removed after several hours, leaving their newly born nymphs behind. These were left in the cabinet until mature. Then they were inspected at intervals of several hours and the newly moulted adults counted and removed until none was left.

EXPERIMENTS AND RESULTS

When batches of adult aphids are kept in the way described, a life table for each individual can be constructed to give the daily nymph production, length of the post-reproductive phase, and length of adult life (see Table I). With experience gained from raising several batches of similar material, an optimum pattern of life-history can be recognized. This is not the mean pattern, for nearly all forms of aberration are one-sided; they reduce the capacity of the individual and lower the mean performance of the group.

There are always some aphids lost during these experiments because their movements are not restricted. Sometimes they are recovered from adjacent plants or the sanded surface of the pots. Also some die. Lost aphids are recorded separately from those found dead, but only after a thorough search has been made to find the corpse. The distinction between "lost" and "dead" is thus largely a matter of behaviour; "lost" amounts mainly to a refusal to accept the plant, although it must still include a few dying aphids which cannot be found. The category "dead" will occasionally include aphids that refused to settle down and wandered until exhausted without leaving the pot. The cause of death or loss in the routine experiments with *A. fabae* is of no consequence for the second category is very small because the host is well suited to the aphid. It becomes important when feeding behaviour in relation to the plant is considered.

Once reproduction has started, it usually continues without interruption until

TABLE I

Life-histories of Aphis fabae *apterous virginoparae, on* Vicia faba

phid No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
ate		Nymphs produced																								
ug.	29	0	5	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	2	0	2	0	0	2	1	0
	30	11	8	11	11	10	10	9	7	10	7	8	9	11	9	7	7	9	10	8	10	10	10	11	11	10
	31	8	9	8	6	7	8	8	8	6	4	8	11	8	9	11	10	10	11	11	10	11	10	7	6	7
ept.	1	8	10	10	11	11	11	10	8	11	10	10	10	13	11	6	7	9	12	10	lost	11	8	11	9	8
	2	10	12	7	11	9	9	5	10	9	7	7	11	11	10	7	12	14	8	10		12	8	9	11	8
	3	9	14	9	8	11	12	9	8	8	9	11	10	12	11	lost	11	10	10	10		10	9	10	11	8
	4	10	11	11	10	9	10	9	10	7	8	11	10	8	10		12	10	9	11		5	9	10	11	11
	5	8	7	7	12	10	10	5	10	7	7	7	8	7	lost		10	13	10	11		7	8	9	8	8
	6	8	9	12	10	9	9	7	7	10	9	7	8	9			4	8	6	10		7	9	9	5	9
	7	11	3	8	5	5	8	6	10	8	6	6	6	9			5	10	7	9		7	6	8	9	11
	8	4	4	7	11	6	2	8	8	6	5	5	3	1			2	4	4	5		5	9	9	6	5
	9	3	2	4	7	2	5	9	7	5	8	3	3	5			2	3	9	4		1	4	6	5	5
	10	3	2	0	2	3	2	2	2	1	2	3	2	2			3	4	4	0		1	4	0	3	2
	11	0	1	dead	1	1	5	1	4	0	2	1	2	0			0	0	1	0		0	2	1	1	3
	12	0	0		1	0	1	0	1	0	0	0	1	3			0	0	0	0		0	0	0	0	0
	13	0	0		0	1	0	0	3	2	0	0	0	0			0	0	0	0		0	0	0	3	0
	14	0	0		1	0	2	0	0	0	1	1	0	1			0	0	0	0		0	0	0	0	0
	15	0	0		0	0	1	2	0	0	0	0	0	0			0	0	0	0		0	0	0	0	0
	16	0	0		0	0	0	0	0	0	0	0	0	0			0	0	0	0		0	0	0	0	0
	17	0	0		0	0	0	0	0	0	0	0	0	0			0	0	0	0		0	0	0	0	0
	18	0	0		0	0	0	0	0	0	0	lost	0	0			0	0	0	0		0	0	0	0	0
	19	0	0		0	0	0	0	0	0	0		0	0			0	0	0	0		0	0	0	0	0
	20	0	0		0	0	0	0	0	0	0		0	0			0	0	0	0		0	0	0	0	0
	21	0	0		0	0	0	0	0	0	0		0	0			0	0	0	0		0	0	0	0	0
	22	0	0		0	0	0	0	0	0	0		0	0			0	0	0	0		0	0	0	0	0
	23	0	0		0	0	0	0	0	0	0		0	0			0	0	0	0		0	0	0	0	0
	24	0	0		0	0	0	0	0	0	0		0	0			0	0	0	0		0	0	0	0	0
	25	dead	0		0	0	0	0	0	0	0		0	0			0	0	0	0		0	0	0	0	0
	26		0		0	0	dead	0	0	0	0		0	0			0	0	0	0		0	0	0	0	0
	27		0		0	0		0	0	0	0		0	0			0	0	0	0		0	0	dead	0	0
	28		0		0	0		0	dead	0	0		0	dead			0	0	lost	dead		0	0		0	dead
	29		0		0	0		0		0	0		0				0	0				0	0		0	
	30		0		0	0		dead		dead	0		dead				0	0				dead	0		dead	
ct.	1		0		0	dead					dead						0	0					dead			
	2		0		dead												0	dead								
	3		dead														dead									
	4																									

ended either by death or because all the available embryos have been produced. In the second instance a long post-reproductive phase normally follows, in common with some other aphids (MITTLER 1958). When the aphid dies before it has delivered all its embryos, nymph production falls in the last two or three days of the aphids' life.

1. Aphis fabae *on a suitable host*

Table I shows part of a series of life-histories for a batch of *A. fabae* collected as 4th instar nymphs from sugar beet (*Beta vulgaris* L.) and white goosefoot (*Chenopodium album* L.) in the field, and transferred to broad beans. This is typical of many such sets of records. Few aphids were lost or died prematurely.

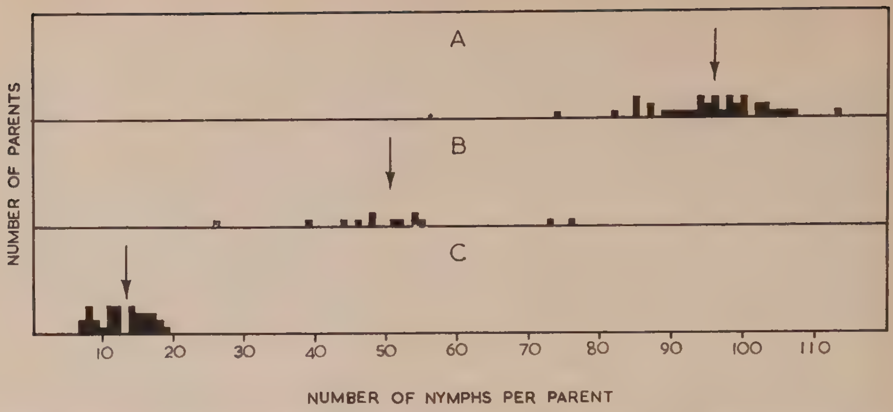

Fig. 1. Total nymph production of: A. *Aphis fabae* on *Vicia faba*; B. Tropaeolum aphid on *Tropaeolum majus*; C. Tropaeolum aphid on *Vicia faba.*

The number of nymphs produced by those which reached the post-reproductive phase is fairly constant (fig. 1A). The host plant is obviously suited to the aphid and the table shows the normal behaviour associated with this. The results for the whole of this batch are summarized in fig. 2 The mean life-histories for aphids found dead and for aphids lost are given separately (parts A & B). Most of the parents appear in fig. 2A, i.e. "dead", but the reproductive pattern for those that were " lost" (fig. 2B) is the same as for those that died, having a long period of reproduction and equally long post-reproductive life. This is a typical diagram for aphids on an acceptable, and suitable, host.

2. Aphis fabae *on an unsuitable host*

When a batch of aphids is presented with an unsuitable host, before reproduction has started, they wander about and finally walk off the plants. They are recorded as lost. Rarely are any nymphs produced, although the aphids contain about 10 or more nymphs virtually ready to be delivered. Fig. 3 shows this behaviour clearly. 27 were 4th instar nymphs and these all remained until they moulted into the adult stage: up to 2 days. Within a few hours of moulting all these aphids left. 21 new adult aphids were also used and these also quickly left the plant; one aphid produced one nymph before leaving. This is a typical example of rejection behaviour and appears in the figure as a pronounced predominance of "lost" over "dead" aphids and almost no reproduction.

3. *Tropaeolum aphid on* Tropaeolum

Fig. 4 summarizes the life-histories of a batch of Tropaeolum aphids kept on *Tropaeolum* leaves. Their life-history pattern is normal for aphids accepting a suitable host; compare with Fig. 2. As mentioned earlier, some of the leaves

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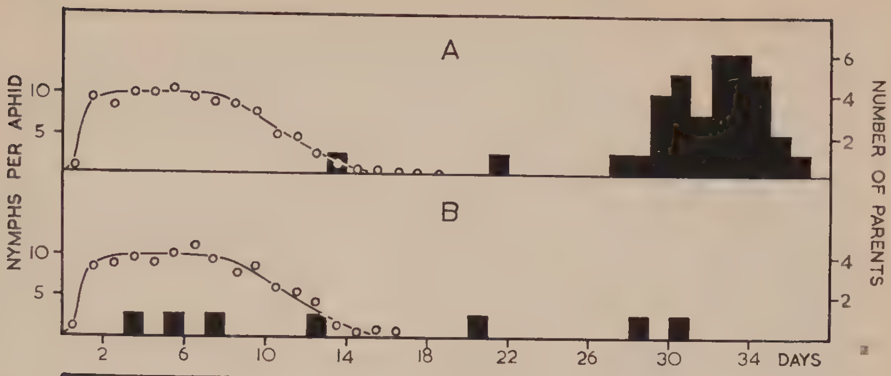
Figs. 2, 3, 4 & 5. Life histories of aphids. A. Aphids that died on the plant. B. Aphids that left the plant.

█ Aphid died or left; ○ Daily nymph production per aphid; 2. *Aphis fabae* on *Vicia faba*; 3. *Aphis fabae* on *Forsythia spectabilis*; 4. Tropaeolum aphid on *Tropaeolum majus*; 5. Tropaeolum aphid on *Vicia faba.*



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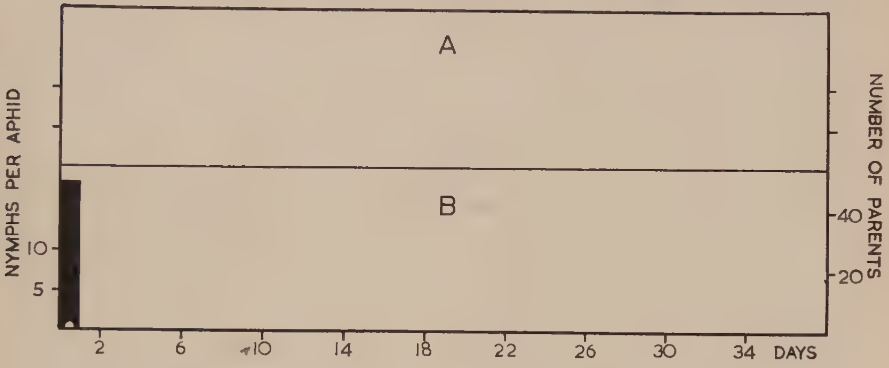
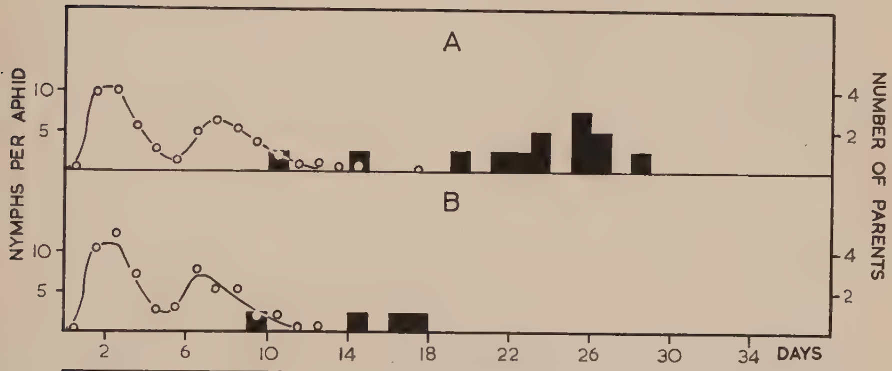


g. 3



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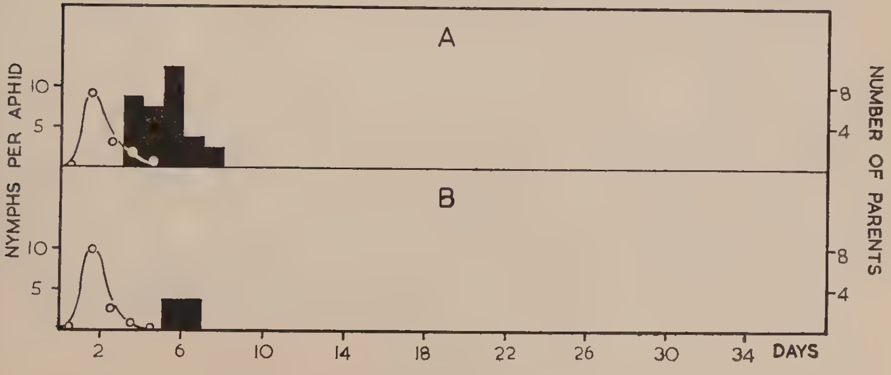

g. 5

Figs. 2—5. Legends on p. 146, under.

used were not fresh; the post-reproduction phase is possibly shortened by this, and nymph production is less regular than in the first example. The number of nymphs per aphid (fig. 1B) is also less, and more variable, than for *A. fabae* on beans.

4. *Tropaeolum aphid on beans*

Fig. 5 shows the life-histories for a batch of the Tropaeolum aphid, put on beans as fourth instar nymphs or newly moulted adults after having been raised on *Tropaeolum majus*. The pattern here is quite distinct from any of the previous examples and is remarkably uniform. All of the aphids settled down exactly as *A. fabae* on beans. The next day they produced a typical number of nymphs but the adults were shrunken; some, which do not appear in this table, were transferred because of this. On the third day nymph production fell abnormally, and, during the next day or so, all except 7 produced those embryos that were already mature (fig. 1C) and died without leaving the plants. The impression gained at the time was that all their feeding responses were satisfied and their behaviour was normal, but they died as if they received no nourishment.

Eight aphids were taken from this batch on the second day of reproduction when they were already shrunken. They were transferred back to their nymphal host *Tropaeolum*. Of these 8, 3 were lost and 1 died. The other 4 revived, recovered their original size, and resumed reproduction. They established healthy colonies. This shows that the batch was initially healthy and did not die from any inherent disability.

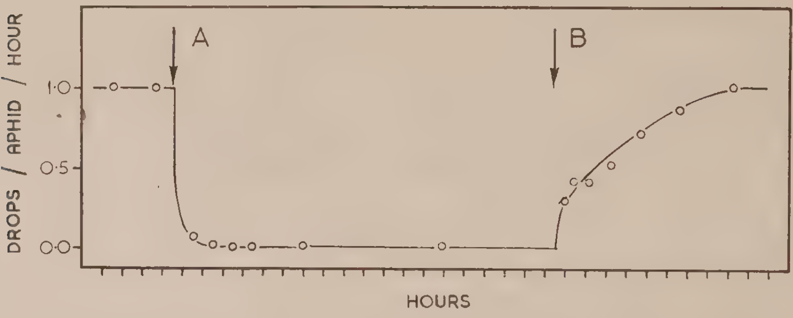

Fig. 6. Hourly rate of excretion of honeydew by a group of Tropaeolum aphids during transfer: A. from *Tropaeolum* to *Vicia;* B. from *Vicia* to *Tropaeolum*.

The revival of aphids returned to *Tropaeolum*, after feeding on beans, indicates that toxic substances are not the major cause of death. The rapid collapse of the aphids on beans suggests that they failed to take up any plant sap. But they could have taken it up and failed to digest it. To study this, the rate of production of honeydew was measured during transfer from *Tropaeolum* to beans and back to *Tropaeolum*; BROADBENT (1951) showed that excretion is mainly dependent upon feeding. Honeydew was collected on bromocresol green indicator paper from a batch of 54 aphids of all stages on *Tropaeolum* at 27° C, 33 % R.H. The aphids were transferred to beans for 19 hours and then to *Tropaeolum*; honeydew was collected throughout this time. The size of droplets was ignored because the aphids were of all sizes.

During the time on beans, 6 aphids died and 4 were lost. After return to *Tropaeolum* a further 7 aphids died and 4 were lost. The rate of droplets produced, per aphid, per hour, is shown in fig. 6. It is quite clear that the aphids took only a little sap during their time on beans. Yet these aphids did not move or withdraw their stylets, and 20 new nymphs were produced during this time.

It is concluded that the aphids died from starvation through either nervous or mechanical inability to feed. It seems unlikely that nervous inability is involved because the behaviour was otherwise normal; but this cannot be determined with certainty from the evidence available. The aphid looks as if it was feeding normally when on beans. This raises interesting questions about the feeding mechanism (see MITTLER, 1957; BANKS & NIXON 1958), which is believed to involve active control by the aphid of phloem pressure forcing sap into its gut, for MÜLLER (1958a) suggests that aphids only remain to feed if their stylets have found the right tissue, yet this aphid stays but receives no plant sap.

To confirm that this aphid will, without coercion, accept beans as a host plant, pots of beans were put about 2 m away from a large colony of Tropaeolum aphids on *Tropaeolum* in a glasshouse. The beans were quickly colonised by large numbers of both apterous and alate aphids. The plants were then removed and kept in another compartment. All the adult aphids started to reproduce, and all died within a few days. The nymphs, newly produced, also died.

The aphids would willingly deposit nymphs on beans, and several attempts were made to raise these nymphs. All failed, although some nymphs moulted once and grew a little in the second instar. Nymphs were raised on excised *Tropaeolum* leaves to compare them with *A. fabae* nymphs. At 20° C, 45 % R.H. and in 16 hours light per day, *A. fabae* on beans took 6.83 days from birth to the final moult; *A. fabae* on *Tropaeolum* took 7.05 days; the Tropaeolum aphid on *Tropaeolum* took 6.54 days. The difference is small, but is probably real; only repeated trials could confirm this.

ANALYSIS OF RESULTS

The aphids in these reproduction experiments have a free choice of feeding site and the subsequent life-history of each individual aphid that remains on a plant is known. Both the feeding behaviour and the hosts' nutritive value to the aphid can therefore be given simple quantitative, though arbitrary, expression. The feeding behaviour can be judged by the *acceptability* of the host, i.e. the percentage proportion of the total aphids that accept the plant offered to them and are eventually found dead on or near it.

The nutritive value, or *suitability*, of the host could be measured by the rate of growth of nymphs, or what is virtually a different measure of the same thing, the rate of reproduction. Reproductive rate is a product of the total fecundity and the length of reproductive life; both of these are affected by nutrition. The mean fecundity of a group of aphids is decreased by poor nutrition and the relative variability between individuals is increased. I have therefore taken the mean fecundity of the group in proportion to the maximum fecundity of any individual as a standard. The length of the post-reproductive phase of life is a more sensitive measure of the state of nutrition of the aphid than the length of reproductive life. I have therefore used twice the post-reproductive period in proportion to the whole life as a standard. The post-reproductive period is multiplied by two because it is never much more than half the total length of life. The product of these two fractions, multiplied by 100, gives a measure of host suitability that varies between 0 and about 100, although it is not a true percentage.

The results are given in Table II. There are no values of host suitability for *A. fabae* on *Forsythia* because all the aphids left; it would be difficult to prove that a host is unsuitable under these circumstances. Apart from this, it is possible to compare the rating given for acceptability with that for suitability of the plant, and the similarity of these two values is a measure of the efficiency with which the behaviour is adapted to the nutritional quality of the host.

TABLE II

The acceptability and suitability of host plants for aphids

				Aphids found dead				
	Number of aphids found dead	Number of aphids lost	Host acceptability (% dead)	Mean fecundity (No. nymphs) $\bar{F}$	Max. fecundity (No. nymphs) F_{max}	Post-reproductive period in days T_p	Total adult life in days T	Host suitability $100.\frac{\bar{F}}{F_{max}}.\frac{2T_p}{T}$
Aphis fabae on *Vicia faba*	36	7	84	95.6	113	16.9	31.9	89
Aphis fabae on *Forsythia intermedia*	0	48	0	—	—	—	—	—
Tropaeolum aphid on *Tropaeolum majus*	13	4	77	50.4	76	11	23	63
Tropaeolum aphid on *Vicia faba*	28	6	83	13.4	19	0.5	5.5	14

The values for *A. fabae* on beans show that both acceptability and suitability were high and they were also well adjusted, (84/89) so that adaptation is almost perfect. *A. fabae* on *Forsythia* has zero for acceptability and suitability is also apparently too low to be measured, hence the aphids' behaviour appears to be adapted to the situation. Tropaeolum aphid on *Tropaeolum* gives a high value for acceptability but only a moderately high value for suitability, evidently reflecting the inferior state of the leaves at some times, hence, although adaptation is not perfect, it is still very good (77/63). In contrast Tropaeolum aphids find beans just as acceptable as *Tropaeolum* but they are quite unsuitable as hosts, hence the efficiency of adaptation of behaviour to the nutritional status of the plant is extremely low (83/14). This completely supports the conclusions drawn from direct observation and inspection of the raw data.

DISCUSSION

The host plant selection behaviour of alate black aphids has been more thoroughly studied than that of the apterae. This is understandable because it are usually the alatae which initially establish, or fail to establish, colonies. Migration by apterae is more limited and the factors that induce it, mainly overcrowding, also often lead to alate production. In *A. fabae* the alate behaviour has been perhaps most completely investigated by JOHNSON (1958). MOERICKE (1955) had already reported the apparent sequence of behaviour patterns involved in flight, settling and colonising.

Stated briefly: alate *A. fabae* in common with many other aphids, will not settle to feed and reproduce without first flying. The change-over, from flight to settling, is complex (KENNEDY 1958) and depends for its effectiveness, among other things, on finding a suitable host. There is a strong reaction against settling down on a non-host plant and this is emphasized by a refusal to reproduce.

Wingless aphids of this, and similar, species, can have no obligatory flight stage. Nevertheless they are much more easily disturbed after the end of the teneral period following the final moult, and before the production of their first nymph, than at any other time. Once disturbed, they often walk about for a long time before settling down again to feed (see also MITTLER 1958), and they seem to be more critical of their feeding site at this time than at others. In fact they give the impression of having an incipient migratory behaviour. If, during this time, they are placed on unsuitable plants they will rarely stay still. This restless behaviour has been commented on many times (e.g. PAINTER 1951, p. 47). It is most prominent before nymph production has started, but it applies to adult aphids on normal host plants short of water at any time (KENNEDY, LAMB & BOOTH 1958); it is remarkable that the aphids in the present experiment did not even respond to an apparent shortage of water.

On aphid resistant plants (i.e. unsuitable hosts), refusal to settle probably leads to the frequent observation that the aphids "died without reproducing" (SCHWARZE & HUBER 1939, JONES 1942). Sometimes there is, in addition to the effect produced by behaviour, a definite reduction in rate of reproduction and rate of growth of nymphs on resistant plants (HARRINGTON 1941). Sometimes growth rate and reproduction together are shown to be affected (MÜLLER 1958b). Most often the total effect of behaviour, growth rate and reproduction are all lumped together in records of colony growth or demise. Rarely is it possible from these kinds of results to know if the aphids were free to choose or refuse the plant during the critical period between the final moult and reproduction. Only when the colony continues to grow effectively through two generations it is possible to infer that the plants were readily accepted.

It is not, therefore, possible to tell how often the kind of behaviour reported here has been observed in aphids. The situation in insects where the parent and offspring have different diets is not strictly comparable. In the present instance with the Tropaeolum aphid on beans, the way in which the parents settled to feed after the final moult, and then went on to deliver their nymphs, is adequate evidence that the host was completely acceptable. The aphids reproduced almost until they died. This is convincing evidence that they were still behaving as they normally do on a host that has been accepted. Yet very little nourishment can have been received by the adults reproducing on beans, for the number of nymphs produced barely exceeds the number already mature in the parent at its final moult. The nymphs feeding on beans must have received some nourishment, for some of them reached the second instar; but again it can only have been a very limited amount.

It is therefore concluded that the behaviour described is not adapted to the aphids' feeding requirements. In an evolutionary sense, the requirements and behaviour are out of step. This may suggest that the aphid described is not a full species, especially in view of STROYAN's (1958) work on hybridity in *Sappaphis*. This point is unlikely to be entirely cleared up until the taxonomy of the group is improved. If the Tropaeolum aphid is a hybrid, it is a regularly produced one, and this kind of unadapted behaviour may be one of the difficulties that hybrids inherently face. If it is a species, this behaviour may suggest its origins in hybridity.

ZUSAMMENFASSUNG

VERFEHLTES ERNÄHRUNGSVERHALTEN BEI EINER SCHWARZEN BLATTLAUS DER APHIS FABAE*-GRUPPE*

Eine *Aphis*-Art der *A. fabae*-Gruppe, die in Südengland häufig an *Tropaeolum majus* zu finden ist, kann von *A. fabae* und ihren nächsten Verwandten durch das lange Endsegment des Rüssels unterschieden werden.

Junge ungeflügelte Imagines von *A. fabae*, die noch nicht zur Fortpflanzung geschritten sind, verhalten sich, wie die Geflügelten, sehr kritisch bei der Wahl der Wirtspflanzen. Auf ungeeigneten Wirten siedeln sie sich weder zur Nahrungsaufnahme noch zum Absetzen von Junglarven an.

Die Lebensabläufe von Blattläusen, die freie Wahl zur Annahme oder Verweigerung ihrer Wirte hatten, wurden verglichen bei *A. fabae* an Ackerbohnen und der *Tropaeolum*-Laus an Kapuzinerkresse und Ackerbohnen.

Es wird gezeigt, daß die *Tropaeolum*-Laus Ackerbohnen leicht annimmt und an ihnen Junge absetzt. Auch Geflügelte versuchen diese Bohnen zu besiedeln. Sowohl Geflügelte wie Larven sterben jedoch an Bohnen ab. Ihr Wirtswahlverhalten ist ihren Nahrungsanforderungen nicht angepaßt.

Bei einem Übertragungsversuch auf Bohnen und zurück auf Kapuzinerkresse zeigen die Exkretionsraten, daß die Blattläuse auf Bohnen keinen Pflanzensaft aufnehmen, obwohl ihre Stechborsten ins Pflanzengewebe eingesenkt bleiben.

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THE INFLUENCE OF DAY-LENGTH ON IMAGINAL DIAPAUSE IN THE RED LOCUST, *NOMADACRIS SEPTEMFASCIATA* (SERV.)

BY

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The main permanent habitats of the Red Locust (*Nomadacris septemfasciata* (Serv.)) in tropical Africa lie between latitudes 7° and 9° S and between 10° and 16° N. Breeding is confined to the rainy season, which occurs during the longest days of the year in these areas. Both north and south of the equator, the adults emerge as day-length is diminishing and undergo a prolonged imaginal diapause until the next rainy season.

When reared in the laboratory in London, adults which emerge in spring or early summer become sexually mature within two months; those which emerge in late summer and autumn, although kept at the same temperature and humidity, enter diapause and do not copulate or oviposit until the following spring. By controlling the length of the daily photoperiod, it has been shown that the seasonal differences in day-length are responsible for the onset of diapause. Constant short photoperiods usually induce a rather shorter diapause than photoperiods diminishing so as to simulate autumn day-length at this latitude (51.5° N). The critical constant photoperiod for the establishment of diapause lies between 12 and 13 hours which corresponds approximately to maximum and minimum day-lengths at 7° N or S. It is therefore possible that day-length is responsible for the establishment of the natural diapause.

The Red Locust, *Nomadacris septemfasciata* (Serv.), has one of its main permanent habitats in the Rukwa Valley in Tanganyika, Central Africa. In this area, which lies approximately between latitudes 7° and 9° South, there is one rainy season lasting from November to March and a dry season lasting from April until October. The locusts, which require moisture for oviposition and egg development and green vegetation for nymphal growth, breed only during the rainy season. The young adults emerge in late February, March and April and have a reproductive diapause of 8 or 9 months lasting until the next rainy season. The diapause prevents breeding during the dry season but is not initiated by drought since, although little rain falls, conditions remain extremely humid for some time after adult emergence and some of the ground remains flooded for several months. Annual fluctuation in day-length at 8° S. amounts to only 55 minutes. The Red Locust also has permanent habitats north of the equator in the flood plains of the Middle Niger and in the neighbourhood of Lake Chad. In these areas, which lie between 10° and 16° North, the seasons are reversed. The rainy season, when breeding occurs, is in July and August and the adults, which emerge in September and October, remain in diapause until the following July. At a latitude of 16° annual fluctuation in day-length reaches two hours. In both northern and southern regions, however, rainfall and breeding occur at the time of the year when day-length is at its maximum and the adults emerge at the time when it is diminishing most rapidly.

In the course of breeding *Nomadacris* in the laboratory of the Anti-Locust Research Centre in London, it was found that while adults which emerged from April to July inclusive matured in from 5 to 9 weeks, those which emerged in the autumn (from about mid-August onwards), although kept at the same temperature and humidity, entered diapause and did not oviposit until the following spring. The body-weight of these adults increased, as in non-diapause adults, by about 50% during the first two or three weeks after emergence, but instead of continuing to increase gradually until maturity it remained virtually stationary for many weeks during the diapause period. The average weight during this period was referred to as the "basic weight". Those adults which emerged in August and September (the "long diapause groups") had a diapause, usually lasting 7 or 8 months, only a little shorter than in natural conditions. Those emerging from October to December (the "short diapause groups") had a rather shorter diapause usually lasting from 4½ to 5½ months. The maturation times of groups of adults emerging from January onwards decreased progressively. They formed a link between the diapause groups and the non-diapause groups which

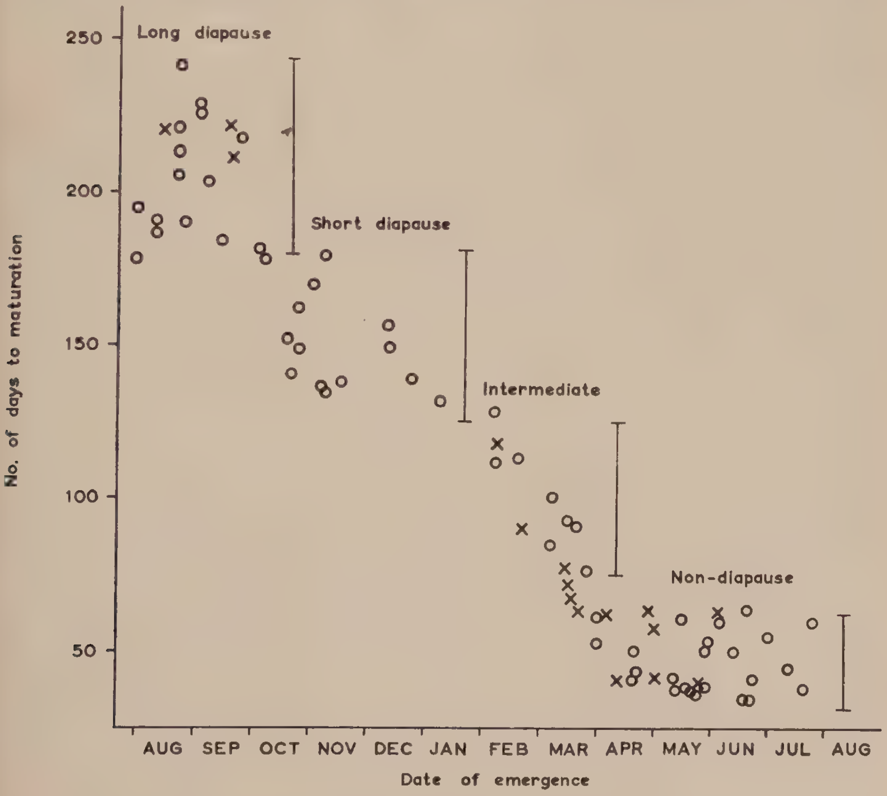

Fig. 1. Maturation times of groups of adults emerging at different seasons of the year (○) or kept in artificial day-length regimes characteristic of the seasons (×).

began to emerge at the beginning of April. These observations have already been published (NORRIS 1959) and the data are summarised here in Fig. 1.

The windows of the breeding-room were unscreened and it was suspected that the seasonal factor responsible for the observed differences in maturation-time must be day-length. This factor had previously been shown to affect the rate of sexual maturation in isolated pairs of *Schistocerca gregaria* (Forskål) (NORRIS 1957). In the experiments described in the following pages the effects of artificial daylengths on the rate of sexual maturation were investigated. A preliminary note on the results of these experiments has been published (NORRIS 1958).

MATERIALS AND METHODS

The locusts used in these experiments were descended from a stock sent to London from Tanganyika in 1953 and bred since then in crowded conditions.

The locusts were kept throughout hopper and adult life in a controlled temperature room maintained at 27 to 28° C. When subjected to artificial day-lengths they were kept in cabinets screened by light-proof curtains and illuminated for the required period by 20-watt fluorescent lamps in the ceiling. The lamps were controlled by Venner time switches. All the adults were kept in groups of from 10 to 12 pairs in cylindrical celluloid cages 38 cm. in height and 20 cm. in diameter. These cages had a total capacity of 12 litres but they were fitted with false floors of perforated zinc under which the sand tubes for egg-laying were inserted, and these reduced the space for the locusts to 9 litres. The hoppers (nymphs) were usually reared in cubical wooden cages of about 50 litres' capacity with glass fronts. In those experiments where the hoppers as well as the adults were exposed to different day-lengths, the hoppers were reared in the 12-litre celluloid cages. The hoppers were reared at high density, about 1000 hatchlings being put into a 50-litre cage in the space of a few days. Their numbers were much reduced by mortality and it was unusual to obtain more than 150 adults from a cage. All the adults emerging in one cage are referred to as a "batch". They were usually set up in the required environment within 24 hours of emergence. Hoppers and adults were fed daily on fresh grass and the hoppers were also provided with dry bran.

The 12-litre cages, whether in the cabinets or in the open room, were kept grouped round 100-watt electric lamps which were switched on for 7 hours daily from 9 am. till 4 pm. (for 3 hours only on Saturdays and not at all on Sundays). These lamps raised the temperature in the centre of the cage to 35 or 36° C and set up a gradient ranging from 32 to 42° C within the cage. The locusts tended to sit in the hottest part of the cage. The large wooden rearing cages had a 60-watt electric lamp fixed to the back wall, and this was switched on for the same number of hours and set up a gradient of about 32° to 50 ° C. Relative humidity was largely determined by the quantity and condition of the grass fed to the locusts and was consequently variable. It was usually between 75% and 61%, falling to between 66% and 42% when the lamps were switched on. It was found that such differences had little effect on maturation-time (NORRIS 1959) but in any one experiment care was taken to maintain approximately the same humidity in the different treatments.

The egg-pods were laid in aluminium tubes measuring 10 cm. long by 4 cm. in diameter. These were re-filled daily with sand freshly moistened in the proportion of 15 ml. of water to 100 ml. of sand.

During the daytime hours when the 100-watt lamps were switched on, the front curtains of the cabinets were rolled up and some of the cages moved so that all those involved in one experiment were kept in the same position, either in the cabinets or on shelves in the open. This ensured that all the locusts were subjected not only to the same temperature but also to the same intensity of both artificial light and daylight. The period when the lamps were on was always shorter than minimum mid-winter day-length and always fell within the hours of daylight. Day-length was therefore determined by the number of hours of daylight or of fluorescent light before or after the hot period and was not affected by the lamps. During the cool hours the maximum intensity of daylight in the lightest part of the room where the locusts were kept varied on a clear day from 22 foot-candles at mid-winter to 37 foot-candles at mid-summer. The fluorescent lights in the cabinets gave an intensity of about 20 foot-candles inside the cages. The 100-watt lamps being placed within a few inches of the cages gave maximum intensities of about 400 foot-candles so that all the locusts were subjected throughout the year to much greater intensity during the hot hours. There was no suggestion that differences in intensity had any effect on maturation-time and most of the work on photoperiodic diapause has shown that the reaction is independent of light intensity provided that intensity is above a very low level, usually about one foot-candle (LEES, 1955), but sometimes even lower. Unless otherwise stated, day-lengths refer here to the period between the official hours of sunrise and sunset as given in the Nautical Almanack (1948). Since maximum intensity near the window was about 2 foot-candles at the official hour of sunset and less than 1 foot-candle about 10 minutes later, this period corresponded fairly well with the period during which the locusts were exposed to light.

THE CRITERION OF MATURITY

A male was considered to be fully mature when it was ready to copulate and a female when it laid its first egg-pod. Ideally, the maturation-time of a group should be the average time taken by all the individuals to reach maturity. The recording of such a figure on a large scale is impracticable because of the labour involved and also because it necessitates an undesirable amount of handling of the locusts. Since, however, there is a high degree of synchronisation within one group and since the interest lies in the relative rates of maturation of different groups, it has been found adequate to record the time taken by the first mated female to lay an egg-pod. Since synchronisation tends to be less perfect in diapause groups than in non-diapause ones the result of this procedure is to minimise rather than exaggerate the differences between them. There is a tendency for the females in diapause groups to mature before the males, and unfertilised egg-pods are occasionally laid by virgin females. The majority of the eggs in such pods do not hatch and since a group is not considered to be mature until at least some of both sexes are mature these pods are disregarded in the figures for maturation time. The question of synchronisation and of the incidence of oviposition by virgin females has been fully considered elsewhere (NORRIS 1959).

THE EFFECT OF ARTIFICIAL SPRING DAY-LENGTH ON DIAPAUSE IN LOCUSTS EMERGING AS ADULTS IN AUTUMN

Experiment I

In this experiment the effect was examined of keeping hoppers and adults emerging in autumn and winter in artificially increasing photoperiods corresponding to early and late spring day-lengths.

The first hoppers hatched on August 25th and were set up at a photoperiod of 8 hours which corresponds to early January day-length in England (sunrise to sunset). The adults emerged on October 7th, by which date the photoperiod in the cages had reached 10 hours 15 minutes (as in late February). Three further batches of hatchlings were set up in the same cabinet in September, October and November respectively and these became adult in November and December at photoperiods corresponding to day-lengths in March, April and early May. The details of these four rearings, with the maturation times of the adults which were set up in groups of ten pairs, are shown in Table I. Two groups which emerged when the photoperiod had reached levels corresponding with February and March day-lengths laid their first pods in 90 and 72 days, respectively. Two groups which emerged at photoperiods corresponding with April and early May day-lengths laid their first pods in 40 and 41 days. All these times are characteristic of true early and late spring groups emerging during the months simulated and they fit perfectly into the curve for seasonal maturation time (see Fig. 1). Some of the adults from the first batch emerging in October were kept in natural winter day-length and these entered diapause, taking 183 days to mature as compared with 90 days for those kept in early spring day-length. Daylight controls were not set up from the other three batches but maturation times of from 40 to 72 days never occur in normal laboratory conditions at this time of the year, and seven unrelated daylight groups emerging during the same autumn and winter took from 139 to 229 days (mean 189 days) to mature.

TABLE I.

Experiment I. Maturation times of 10-pair groups reared during the autumn and winter months in artificial day-lengths gradually increasing as in spring. (Hoppers as well as adults in increasing photoperiods).

Group	Date hatched	Date adults emerged	Photoperiod at emergence hours and minutes	Corresponding spring date (Sunrise to sunset)	Maturation time *
K	25/8	7/10	10.15	20/2	90
M	21/9	2/11	11.39	13/3	72
N	24/10	2/12	13.30	10/4	40
Q	10/11	22/12	14.47	1/5	41

* days from emergence of adults until deposition of the first pod.

Experiment II

This experiment differed from the previous one in that all the locusts were reared as hoppers in natural autumn or winter daylight and the transfer to artificial spring or summer conditions was made only at the beginning of adult life. From each batch one group of ten pairs was kept in daylight[1]) and the other in artificial spring or summer day-length. Five batches of adults were involved, the first emerging on October 5th and the last on December 21st. The first two batches were kept in day-lengths corresponding to those in May and June and the last three to those from April to early May. The results are shown in Table II.

TABLE II.

Experiment II. Maturation times of 10-pair groups of adults kept in natural autumn and winter daylight and in artificial day-lengths gradually increasing as in spring. (Hoppers all reared in daylight).

	Daylight groups		Artificial "Spring" groups			
Date of emergence	Group ref.	Maturation time (days)	Group ref.	Photoperiod at emergence (hours & minutes)	Corresponding spring date (sunrise to sunset)	Maturation time (days)
5/10	S	188	R	16.0	22/5	41
23/10	C	182	D	16.22	5/6	65
9/11	X	142	W	13.0	3/4	63
8/12	II	168	I	14.36	27/4	65
21/12	P I	139	P II	14.45	1/5	57

The maturation times of the groups kept in winter daylight varied from 139 to 188 days and those of the groups kept in artificial spring day-length from 41 to 65 days. Diapause is therefore prevented by the action of spring day-length on the adult stage alone. Three out of the five artificial day-length groups took from 63 to 65 days to mature which, although within the range of maturation time of true spring groups, is near the upper limits.

THE EFFECT OF ARTIFICIAL SPRING AND AUTUMN DAY-LENGTHS ON DIAPAUSE IN LOCUSTS EMERGING AS ADULTS IN SUMMER

Experiment III

Two artificially lit cabinets were used simultaneously in this experiment, a "spring" cabinet in which photoperiod started at 9 hours as in early February and increased as in spring, and an "autumn" cabinet in which it started at 15 hours as in early August and decreased as in autumn. Hoppers hatching on June 17th were divided into three groups, one kept in the spring cabinet, one kept in the autumn cabinet, and one kept in natural summer day-length beginning at its maximum of 16 hr. 38 min. The adults emerged at the end of July, by which time day-length was 11 hr. 45 min. in the spring cabinet (mid-March) and 12 hr. 15 min. in the autumn cabinet (mid-September); natural day-length was 15½ hours at this time. Day-length in the spring cabinet was therefore still slightly shorter than in the autumn cabinet at the time of emergence and the position was not reversed until the adults were nearly a week old. From the three batches of newly emerged adults, 6 ten-pair groups were set up as shown in Table III which also shows their maturation times. It will be seen that the groups kept in autumn day-length, whether reared as hoppers in autumn day-length or in summer daylight, both had the long diapause characteristic of adults emerging in September, while those kept in spring day-length matured without diapause whether reared as hoppers in spring day-length, autumn day-length or natural summer daylight. The one group both reared and kept as adults in summer daylight matured slightly more quickly than the other non-diapause groups, and this was probably due to the fact that the artificial spring day-length started at the mid-March level of 11 hr. 45 min. The most rapid, non-diapause maturation is not shown by adults emerging before the beginning of April (see fig. 1).

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[1]) The term "daylight" is used here to indicate natural seasonal day-length in London (at 51.5° N); no reference to the quality or intensity of the light is implied.

TABLE III.

Experiment III. Maturation times of 10-pair groups of Nomadacris *kept in artificial spring and autumn day-lengths and in summer daylight. (Hoppers reared in one of these three regimes).*

Rearing daylength		Days until maturation
Hoppers	Adults	
Spring	Spring	69
Autumn	Spring	64
Summer (natural day-length)	Spring	76
Autumn	Autumn	223
Summer (natural day-length)	Autumn	209
Summer (natural day-length)	Summer (natural day-length)	59

Experiment IV

This experiment was carried out in the "spring" and "autumn" cabinets at the same time as the previous one, but it was begun a week later; the locusts were exposed to artificial spring and autumn day-length during adult life only. Adults reared in daylight and emerging at the end of June were set up in three groups, one kept in daylight, one in the spring cabinet and the other in the autumn cabinet. The daylight group matured, as is usual in summer, without diapause in 54 days. The spring and autumn groups matured, respectively, in 110 and 220 days, times typical of adults emerging in February and August respectively (see fig. 1). A few females in the autumn group escaped diapause and laid parthenogenetically at various times during the "winter". As previously recorded (NORRIS 1959) such females sometimes occur in the earliest diapause groups emerging in August. Full response to autumn and winter day-lengths was therefore induced by exposing the adults only to them.

The foregoing experiments show conclusively that the winter diapause which occurs *in this laboratory* is attributable to the seasonal change in day-length operating during adult life.

THE DURATION AND TERMINATION OF DIAPAUSE

The observation that adults emerging in August and September have a longer diapause than those emerging later in the year suggested that the diapause was being cut short by the increasing day-length in spring. However it was also observed that the post-diapause resumption of weight increase which anticipates maturation often occurs as early as mid-January, before any appreciable increase in day-length has occurred. Oviposition may actually begin in late February or early March when day-length has reached only 11 hours. The only other environmental factor which could cut short diapause would be a change in the composition of the grass on which the locusts are fed. This is very unlikely since the nature of the grass during the early months of the year is exceedingly variable according to the severity of the winter. In mild winters it may remain fairly succulent throughout and in others it may be in very poor condition owing to frost damage. In the following experiment the effect of depriving diapausing adults of increasing day-length after the shortest day was examined.

Experiment V

From each of four batches of adults emerging on August 16th, August 22nd, November 6th and November 8th two groups of adults were set up. One group from each batch was kept in daylight throughout and the other was kept in daylight until December 21st, after which it was screened from the subsequent increase in day-length. Maturation times of the four daylight groups in order of emergence were respectively 205, 190, 179 and 144 days. Those of the four screened groups were respectively 227, 190, 177 and 150 days. Any effects of screening after December 21st were therefore negligible.

The above experiment showed that the lengthening days of late winter are not responsible for the onset of maturation either in the "long" or the "short-diapause" groups (see p. 155). It was nevertheless suspected that the shorter and progressively diminishing maturation times of the "intermediate" groups emerging after December were attributable to the longer days of spring. The maturation times of these groups form a link between those of the "short-diapause" groups emerging from October to December and those of the non-diapause groups emerging from April to July. The following experiment was designed to investigate this question.

Experiment VI

Two groups of ten pairs of adults were set up from each of six batches of locusts emerging from October to March. One group from each batch was kept in daylight and the other in constant short day-lengths of 8 or 11 hours. (Two constant short-day groups, one at 8 and one at 11 hours, were set up from one batch emerging in March). The results are shown in Table IV. All the constant short-day groups, whether kept in 8- or 11-hour day-lengths and whether they emerged before or after December, had very similar maturation times of about 5 months. The "short-diapause" daylight groups emerging from October to December matured in almost the same time as their parallel short-day groups and were clearly responding as to constant short-day conditions. The "intermediate" daylight groups emerging in February or March, on the other hand, all matured more rapidly than their parallel short-day groups, the degree of acceleration being greater the later in the year they emerged.

TABLE IV.

Experiment VI. Maturation times of adults kept in winter and early spring daylight compared with those of adults kept in constant day-lengths of 8 or 11 hours.

Batch	Date adults emerged	Daylight Group		Constant Short-day Group	
		Approx. at emergence day-length	times (days) Maturation	Photoperiod (hrs. per day)	Maturation time
1	17/10	10½ hrs.	152	11	159
2	16/11	8¾ ,,	139	8	139
3	10/12	8 ,,	148	8	145
4	6/2	9¼ ,,	110	8	160
5	14/3	11¾ ,,	92	8	163
				11	154
6	26/3	12½ ,,	77	11	166

The inference to be drawn from the results of the last two experiments is that the long days end or prevent diapause if they reach the required level early enough in adult life, but that in continuing short-day conditions there is a maximum term to the length of the diapause after the expiry of which maturation occurs independently of changing day-length. Day-length rises to 13 hours at the beginning of April so that groups emerging in the autumn do not experience even 13-hour days until they are several months old and are unaffected by the longer days because these are forestalled by other influences. Groups emerging in early spring, on the other hand, experience 13-hours days at an earlier stage, the later ones very soon after the locusts have reached basic weight. It is probable that diapause does not really become established in these spring groups, but the following experiment shows that even in the "long diapause" groups the diapause can be broken by early transfer to long-day conditions.

Experiment VII

One of two groups from the same batch which emerged in late August was kept in autumn daylight and the other at a constant photoperiod of 11 hours. By November, diapause was well established, the weight of the locusts in both groups having been stationary for several weeks. On November 11th they were transferred to a long photoperiod of 15 hours. One week later the weight of the females began to rise slowly and the first pods were laid in the two groups on Dec. 31st and Jan. 9th, seven and eight weeks after the transfer. In both groups copulation was observed just before the first oviposition. A third group from the same batch was kept throughout in winter daylight and did not mature until the end of February; this was unusually early for a long diapause group, but even so maturation was accelerated by nearly two months as a result of transfer to long-day conditions at the age of 2½ months. The resumption of weight increase in the females occurred about two months later in the daylight group than in the other two groups and all the groups took about the same time to mature after this point was reached. Transfer to long-day conditions, therefore, cut short the diapause but did not accelerate post-diapause development.

The uniformity in maturation time of the constant short-day groups, whether they emerge in autumn or spring, does not suggest that there is any accelerating spring factor other than day-length to account for the earlier maturation of the "intermediate" groups. Providing day-length remains constant, the maturation processes of adults emerging in the early spring take the same time as those of adults emerging in the autumn. There is therefore no reason to suspect the influence of any other factor such as spring grass or increased intensity of daylight. The constant short-day groups were fed on the same grass and exposed to the same light intensity during the brightest hours of the day as their parallel daylight groups. This strongly confirms the conclusion that the termination of diapause under short-day conditions is the result not of any change in the environment but rather of some internal stimulus. Such a stimulus may be provided by the accumulation up to a certain required level of a maturation hormone, a process which might be expected to proceed at a uniform rate under constant environmental conditions but which might well take longer to complete in a cooler or drier environment.

There remains to be explained the fact that the locusts emerging in August and September have a longer diapause than those emerging later in the autumn. These groups are exposed to long days during nymphal and early adult life, but day-length falls rapidly to below 12 hours before they are six weeks old. It seems possible that it is the actual *change* in the level of day-length which has the effect of prolonging the time required for diapause development. Experiments on the effects of changing day-lengths are at present in progress. Insects usually respond to absolute levels of day-length rather than to change (LEES 1955) but CORBET (1956) has shown that metamorphosis in the dragonfly *Anax imperator* is stimulated by increasing spring day-length although not by constant long-day conditions.

THE CRITICAL PHOTOPERIOD

It has already been recorded (NORRIS 1958) that when the photoperiod is maintained at a constant length, the critical level for the induction of short diapause lies somewhere between 11 and 13½ hours. The extremes of day-length which occur in London (7 hr. 50 min. to 16 hr. 38 min.) therefore lie well outside the critical field and need not be considered here. Day-length (from sunrise to sunset) at 7° S. which is the latitude of the northern or equatorial end of the Rukwa Valley, Tanganyika, fluctuates by only 50 minutes, from 11 hr. 40 min. to 12 hr. 30 min. It is possible that day-length in the Rukwa Valley and throughout the equatorial area is always below the level which permits non-diapause maturation. If, on the other hand, maturation in this area is influenced by the changes in day-length, the locusts must be responsive to very small deviations from a photoperiod of about 12 hours (or possibly 12½ hours if twilight periods are included) and the limits of the critical or transitional field must be very narrow. In March, when the majority of adults emerge, day-length is just over 12 hours, and it first falls below 12 hours at the beginning of April. During April it decreases at the maximum rate of about half-a-minute per day and it does not again exceed 12 hours until the beginning of October when the adults are about 7 months old and the rainy season is approaching.

Experiments designed to establish more exactly the limits of the critical constant photoperiod for the induction of short diapause are in progress. They are as yet incomplete, but the first results are shown in Table V. In these experiments comparisons were made between the maturation times of groups of adults subjected to constant photoperiods of 12 or 13 hours and those of groups from the same batch subjected to longer or shorter photoperiods or to natural spring day-length. Three groups exposed to a 12-hour photoperiod all had a short diapause and only one of them matured appreciably more rapidly than the group from the same batch exposed to an 11-hour photoperiod. Three groups exposed to a 13-hour photoperiod all matured without diapause; their maturation times were near or slightly above the upper limit for summer daylight groups, but so far no groups kept at even the longest constant photoperiods have matured as rapidly as the earliest daylight groups. The body-weight of the females increased without interruption from emergence until maturation. Further experiments are required, particularly those in which locusts from the same batch are exposed to both 12- and 13-hour photoperiods. It is already evident, however, that the greatest difference in response does occur between these levels which correspond with minimum and maximum day-lengths (including twilights) in the permanent habitats nearest to the equator.

TABLE V.

Maturation times of groups from the same batch subjected to constant photoperiods of different lengths or to spring daylight.

Batch	Date adults emerged	Photoperiod (hours)						
		8	11	12	13	14	16	Daylight
A	17/11	139			65		62	
B	30/1		141	138	74			
C	8/9		188	198	61	59		
D	9/5		174	131				41
E	27/3		166	158				77

A further possibility which is being explored is that the effects of photoperiods in the neighbourhood of 12 and 13 hours are intensified if they are slowly increasing or decreasing as in natural conditions. In view of the very small rate of change in the natural habitat this seems unlikely, but it is a possibility which cannot be excluded. As previously mentioned, it is suspected that rapidly decreasing autumn day-length in England may be responsible for prolonging winter diapause in the laboratory.

THE EFFECT OF TOTAL DARKNESS

It has often been found in insects that diapause induced by short days does not occur when the insects are kept in total darkness (LEES, 1955). The following experiment showed that this is the case in *Nomadacris.*

Experiment VIII

Three groups of adults from the same batch which emerged in May were set up in daylight, in total darkness, and at a constant photoperiod of 11 hours. The daylight group matured in 41 days and the group kept in darkness in 43 days.

Copulation was first seen in both groups on the same day. The 11-hour group was still immature at the age of 6 months.

DISCUSSION

The occurrence of imaginal diapause in response to short days would certainly seen to be of adaptive value to an insect which inhabits areas in which a season of drought unsuitable for breeding coincides with the shortest days of the year. Such conditions exist in the permanent habitats of the Red Locust both north and south of the equator. Since wet conditions still prevail for some time after the adults emerge, some explanation is required for the onset of diapause. It does not seem at all probable that low temperature is responsible for failure to become sexually mature. Temperature in the Rukwa Valley in Tanganyika is lower from April to August than during the breeding season, but the mean maximum screen temperature does not fall below 30° C. nor the mean minimum below 15° C. (CHAPMAN 1957). Microclimatic observations by BURNETT (1951) showed, moreover, that midday temperature at the top of the grass during April exceeded 38° C. RAINEY, WALOFF & BURNETT (1957) have recorded that *Nomadacris* body temperatures greatly exceeded air temperature and reached 35 to 40° C. at midday in June.

Recently, in London, a group of adults emerging in autumn was kept at ordinary room temperature fluctuating between 17° C. and 22° C. and raised to about 32° C. for only 7 hours daily. It was subjected to a 16-hour photoperiod and matured rather slowly but without diapause in 94 days. This shows that the continuously high temperature of 28° C. used in these experiments is not necessary for non-diapause maturation provided that day-length is adequate. The critical level of photoperiod for the induction of diapause in the laboratory has been shown to lie between 12 and 13 hours, and day-length in the field falls below 12 hours (or 12½ hours including twilights) soon after the wild adults emerge. There is therefore good reason to suppose that the diapause is initiated by day-length. Final conclusions as to the exact manner in which day-length operates must, however, await the results of further experiments, which are at present in progress, on the effects of constant and changing photoperiods corresponding with day-lengths experienced in the natural habitat. It is uncertain if maturation eventually occurs in response to the increasing day-length after the shortest day or if it occurs, as it does in the laboratory, independently of day-length. The laboratory adults take on the average about five months to mature when subjected to constant short day-length. The wild adults, on the other hand, take 8 or 9 months to mature. In the laboratory, temperature and humidity are at all times favourable for rapid maturation. The difference in the duration of diapause may be simply attributable to an accelerating effect of constant high temperature on the physiological changes which occur during diapause or on post-diapause development. But it is also possible that in the field the dry conditions prevailing during the latter part of the diapause period may inhibit or retard maturation. Although these conditions do not prevail in time to initiate the diapause, the association of oviposition with the onset of the rainy season does suggest that they have the effect of prolonging the diapause beyond the point where, in a more favourable environment, the maturation processes would be resumed.

It would be of great interest to know at what time the first signs of approaching maturation occur in the field. Robertson (1958) has made dissections of field samples which showed that yolky eggs between 2 and 3 mm. long sometimes appear in the ovaries before any rain has fallen. It is, however, doubtful if observations were started early enough to detect the point at which yolk first appears in the eggs, which in laboratory adults usually occurs about two months before oviposition, when the eggs are still only 1 mm. in length. The growth of the eggs is, moreover, preceded by the resumption of weight increase in the females and it is this which first marks the turning point in the life-history. Chapman (1957) studied the level of feeding by inspection of the gut contents throughout adult life and concluded that feeding was at its lowest in August and September. If so, it is improbable that weight increase is resumed before October. Regular weighings of samples would, however, provide a more accurate index.

The observations of Albrecht (1953) suggest that in the field diapause may be prevented or abbreviated by high humidity. Adults emerging at the end of March (that is, during the normal period of emergence) were kept in outdoor cages in very humid conditions (70 to 90% relative humidity). The first pod was laid after 100 days and mass laying started in August; exact dates are not available but this means that the majority laid after about four months. The time taken was similar to that taken by the most rapid "short-diapause" groups in the laboratory but it cannot be said whether or not these locusts had a diapause. Development was slow, but since night temperatures were low, it may have been continuous as in the cold laboratory group mentioned on p. 165. The progeny of these locusts emerged as adults in mid-November at the normal field breeding season. They were again kept in outdoor cages and the first pod was laid after 30 days, a time slightly shorter than that of the earliest non-diapause group in the laboratory. Both generations of adults were kept at high humidities, but temperatures were higher for the second than for the first. While the very rapid maturation of the second generation might, therefore, be attributed to temperature, it is quite possible that day-length was at least partly responsible for it. As previously recorded (Norris 1959), attempts to break the long winter diapause in the laboratory by high humidity have not met with success, but this may have been because day-length was too far below the critical level.

Day-length has been shown to play an important part in regulating the life-cycles of many insects, and the work on this subject has been reviewed by Lees (1955). So far as has been ascertained, such effects have not hitherto been demonstrated in the case of an insect (or indeed of any animal) the distribution of which is confined as is that of the Red Locust to tropical regions where fluctuation in day-length is very small. Critical photoperiods are, however, sometimes very short and there is no *a priori* reason why a fluctuation of 50 minutes should not influence diapause. Dickson (1949), for instance, showed that when larvae of the Oriental Fruit Moth (*Grapholitha molesta*) were kept at a temperature of C., 96% of the larvae entered diapause in response to a photoperiod of 13 hours and only 4% to one of 14 hours. A much greater body of work exists on the effects of day-length on plants and here, there are examples of remarkable sensitivity on the part of tropical species. Jagoe (1952) showed for instance that when some varieties of rice are grown in Malaya between latitudes 2° and 6° N. they show seasonal differences in flowering time which are correlated with latitude. Flowering occurred only during the shortest days from October to February. Fluctuation in day-length at 3° N. amounts to 20 minutes and when at this latitude day-length was artificially maintained at the maximum June level the flowering of plants sown in June was delayed as compared with that of daylight controls.

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RESUME

L'INFLUENCE DE LA LONGUEUR DU JOUR SUR LA DIAPAUSE IMAGINALE CHEZ LE CRIQUET ROUGE

On a déjà constaté que, quand on élève le criquet rouge, *Nomadacris septemfasciata* (Serv.), au laboratoire à Londres, les adultes, malgré le fait qu'on les retient dans une ambiance très constante, montrent de grandes différences saisonnières dans la durie du développement qui conduit à la maturité sexuelle. Les adultes qui émergent à partir du mois d'avril jusque'au mois de juillet deviennent matures sexuellement sans diapause en 1½ à 2 mois; tandis que ceux qui émergent au début de l'automne, à partir de la mi-août approximativement jusqu'à la fin de septembre, ont une diapause prolongée de 7 à 8 mois; et ceux qui émergent encore plus tard dans l'année, du mois d'octobre jusqu'à mi-décembre, ont une diapause plus courte de 4½ à 5½ mois. Les périodes de maturation des adultes qui émergent à partir de janvier et plus tard, diminuent progressivement, en se fondant dans les groupes des "non-diapause", qui commencent à émerger au début d'avril. Les expériences exposées dans cette thèse indiquent que les différences saisonnières de la longueur des jours, sont responsables des temps de maturation.

Certains groupes de criquets, émergeant comme adultes vers la fin de l'été ou en automne, ont été soumis à des journées de longueur artificielle qui correspondait à la durée des jours de printems qui n'exerce son influence que sur la vie adulte, suffit à produire ce dans les délais semblables à ceux des groupes qui émergent au printemps. La longueur des jours de printemps qui n'excerce son influence que sur la vie adulte, suffit à produire ce résultat.

Quelques larves étaient élevées simultanément dans les conditions d'une durée artificielle des jours du printemps, d'une durée artificielle des jours d'automne, et d'une lumière naturelle des jours d'été à Londres. Les adultes qui avaient émergé au mois de juin étaient ou maintenus dans le même régime de longueur de journée que les larves, ou transférés dans une des autres conditions. Tous les temps de maturation étaient appropriés aux saisons qui correspondaient au régime de durée du jour auquel les adultes étaient retenus, et il n'y avait nulle évidence qu'ils étaient influencés par la durée des jours pendant leur vie larvaire.

Les groupes "diapause", quand ils étaient soumis à la lumière normale du laboratoire, sont devenus matures, au printemps, au moment où les jours s'allongeaient. Pourtant la maturation n'est pas due à la stimulation causée par un accroissement de la longueur du jour, car on à observé qu'elle arrive en même temps si les criquets ne la subissent pas. Ceci est vrai également à l'égard des deux groupes, celui "de longue diapause" (qui émerge au début de l'automne) et celui "de courte diapause" (qui émerge vers la fin de l'automne); il s'ensuit que la diapause plus courte du dernier groupe n'est pas attribuable, comme il semblait probable, au fait qu'on l'avait exposé à la longueur des jours du printemps plus tôt dans la vie adulte. On a trouvé que ces groupes de la dernière partie de l'automne étaient devenus matures dans le même temps s'ils étaient retenus aux courtes photo-périodes constantes de 8 ou 11 heures, ou à la durée changeante des jours d'hiver. On a conclu qu'ils répondent comme à la durée de constants jours courts.

Les groupes "intermédaires" (qui émergent à partir de janvier jusqu'a mars) d'autre part, sont exposés aux jours plus longs du printemps et de l'été encore plus tôt dans la vie

adulte, et dans leur cas la diapause est empêchée, ou au moins abrégée, par les jours plus longs. Quand ces groupes sont retenus aux constantes photo-périodes courtes, ils deviennent matures plus lentement que s'ils étaient maintenus à la lumière du jour, et ils ont une diapause de la même longueur que les groupes de courte-diapause.

On a conclu de ces résultats que, quand les groupes sont retenus dans conditions de jours-courts, les processus de maturation (manifestés par un accroissement du poids du corps, et par le développement de l'ovaire) sont repris à un certain stade indépendamment de la longueur du jour et sous l'action de stimuli internes. L'exposition aux conditions de jours longs n'est effective que pour accélérer la maturation si elle a lieu avant que ce stade soit atteint.

L'observation que les adultes émergeant au début de l'automne ont une diapause plus longue que ceux qui émergent vers la fin de l'automne, reste sans explication. Ceux-là sont exposés à la durée de jours qui décroissent rapidement pendant la vie larvaire et du commencement de la vie adulte, et il semble probable que le changement propre des jours longs aux jours courts puisse amener une diapause plus longue que ne le feraient des jours courts dès le début. A présent, on étudie les effets de la variation de durée des jours.

Les adultes retenus dans l'obscurité constante sont devenus matures sans diapause.

La grande fluctuation de la longueur des jours, caractéristique de la latitude de Londres, n'est pas nécessaire pour induire les deux types de maturation "diapause" ou "non-diapause". Les plus grandes différences de réponse aux photo-périodes constantes ont eu lieu entre les moyennes correspondant à peu près avec les minimum et maximum de longueurs de jour (y compris les périodes de crépuscule) dans les habitats tropicaux du criquet.

Il est discuté ici de la probabilité que le cours de la diapause dans l'habitat tropical naturel soit influencé par la faible fluctuation des longueurs de jour de cet habitat.

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K. MARAMOROSCH : *Ephemeral disease of maize*

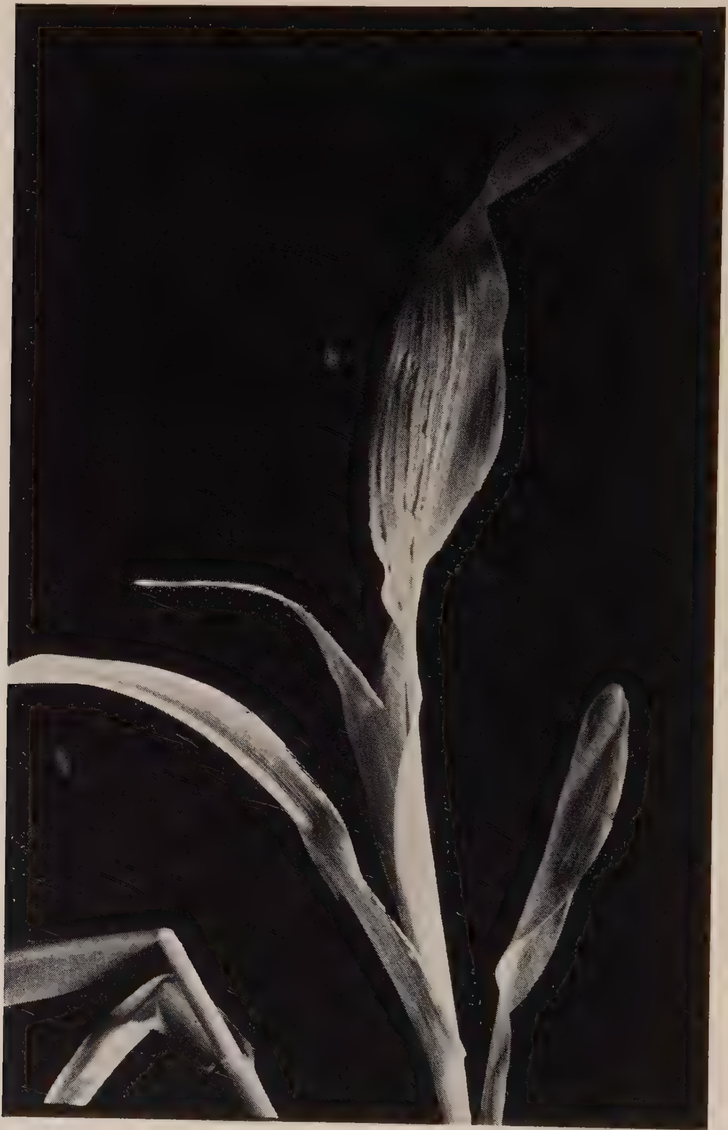

Fig. 1.

Section of diseased leaf of *Zea mays* with enations caused by the feeding of *D. elimatus* nymphs (Photograph by J. A. CARLILE).

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SHORT COMMUNICATION

AN EPHEMERAL DISEASE OF MAIZE TRANSMITTED BY *DALBULUS ELIMATUS*

BY

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Wallaby-ear disease of maize was described from Queensland and other parts of Australia (SCHINDLER, 1942; VEITCH, 1942—43). The leafhopper *Cicadulina bimaculata* Evans was described as the vector. Recent investigations in Australia (DAY, personal communication) have indicated that the progeny of infective *C. bimaculata*, without having fed on diseased plants, can cause the disease. Several plant viruses are known to pass through the egg of infective females to their progeny and the infective agent of the Wallaby-ear disease may belong to that group. Most workers in the field seem to have accepted the view that Wallaby-ear disease is caused by a virus, but the viral etiology has never been proved crucially. Preliminary observations presented below may have a bearing on this problem. The observations deal with a maize disorder new to the Northern Hemisphere and strikingly similar in its signs to Wallaby-ear disease.

During a study of maize diseases in Mexico in the summer of 1955 (MARAMOROSCH, 1958) leafhoppers of the species *Dalbulus elimatus* DeL. & W. were collected near Chapingo. A colony of these leafhoppers was maintained on corn plants in a greenhouse. In one of the tests a small number of hybrid sweet corn (maize) plants of the variety Golden Bantam were exposed to the feeding of leafhopper nymphs. Striking swellings developed along the secondary veins on the underside of the youngest leaves of two plants which were colonized with 30 nymphs for 7 days. The top leaves of one plant and a single leaf of the other one (Fig. 1) showed pronounced distortions; only a few tiny swellings were noticed on another leaf. The symptoms did not become systemic. After removal of the insects the swelling continued to increase in the affected veins, reaching a maximum on the 12th day, by which time the entire veins were protruding as a result of a fusion of the separate swellings. Afterwards the swellings decreased gradually and on the 25th day the leaves showed no signs of disease and the plants could not be distinguished from healthy controls. The exposed plants were kept under observation for several weeks; no stunting or other disease signs were noted.

The nymphs which induced the enlarged veins molted and became adults. Six of these adults were tested individually on 25 maize plants of the variety Golden Bantam. However, only one of 25 plants showed slightly enlarged veins.

Adults and nymphs of *D. elimatus* and of the closely related species *D. maidis* Del. & W. both known vectors of stunt virus (NIEDERHAUSER & CERVANTES, 1950), were caged on the two maize plants with diseased leaves for 5 days, in an attempt to acquire the causative agent and to transmit it to fresh plants. Eighteen individuals of each species were tested; a total of 87 plants were exposed to *D. elimatus* and 75 plants were fed on by *D. maidis*. However, no transmission was obtained with either species.

The affected leaves of the two maize plants were shown to a number of virus workers and al noticed the striking resemblance to Wallaby-ear disease. Workers from Australia, who had firsthand experience with Wallaby-ear, stated that the swellings most closely resemble the Australian disorder. The Australian disease seems of little economic importance because the signs tend to disappear and no appreciable decrease in yield has been found. In this respect the newly found disease of maize seems to resemble the Australian syndrome.

The lack of systemic infection, the gradual disappearance of signs followed by complete recovery, and the inability of insects to repeat the performance, as well as the failure of *D. elimatus* to acquire an infective agent from the affected leaves strongly suggest that the disease was caused by an insect toxin, rather than by a virus. Whether or not the Australian disease is of similar nature to the disease resulting from the feeding of *D. elimatus* nymphs can not be stated at present.

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ZUSAMMENFASSUNG

EINE VON DALBULUS ELIMATUS *ERZEUGTE MAISKRANKHEIT, DIE DER ADERNVERDICKUNG DES MAISES ÄHNELT*

Bei Untersuchungen von Maiskrankheiten in Mexiko (1955) erzeugten Larven der Maiszwergzikade, *Dalbulus elimatus* DeL. & W., an Maishybriden der Sorte Golden Bantam auf den Blättern Aderanschwellungen, die Symptomen der Adernverdickung des Maises außerordentlich ähnelten. Diese ist bisher nur in Australien bekannt und wird von *Cicadulina bimaculata* Evans(auch transovarial !) übertragen, ist jedoch virologisch noch nicht näher analysiert. Die Symptome wurden jedoch nicht systemisch, ließen sich weder von *D. elimatus* noch von der verwandten *D. maidis* DeL. & W. weiter übertragen und verschwanden nach einiger Zeit wieder völlig. Sie beruhen vermutlich eher auf einem Insektentoxin als auf einem Virus.

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FEEDING PERIOD, GROWTH, AND PUPATION IN LARVAE OF *DROSOPHILA MELANOGASTER*

BY

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Food uptake of *Drosophila melanogaster* larvae was limited by removing larvae from the food after different periods. Some of these larvae were transferred to culture jars without food to study further development. The minimal feeding period necessary to complete development was 48 hours at 25°C; the larvae must have reached the third instar. Mortality of larvae with feeding periods of 48 hours or more was low. Puparium formation was retarded only in larvae with a feeding period of 48 hours. Sex ratio of emerging adults was not influenced by underfeeding. Weight of adults was taken twice: alive and dried. Females were always somewhat heavier than males. Underfeeding may produce adults with weights only $^1/_4$ to $^1/_5$ of the normal weight.

The larvae removed from the food at different intervals were weighed alive and dried, and a growth curve was thus constructed. Increase in live weight was observed until 72 hours after hatching, whereas dry weight reached its maximum after 84 hours. The difference is due to water loss preceding puparium formation. During the pupal stage a slight decrease in dry weight is noted, presumably caused by metabolism. During the starvation period between removal from the food and puparium formation, a loss of weight is found which depends on the duration of the starvation period.

If an insect is to complete its development it must take up a certain minimum amount of food, and it must take it up at a certain minimum rate. There most certainly exists some relation between these two quantities, which depends also upon the energy expended to obtain the necessary food.

The amount of food necessary can be studied by supplying a number of individual larvae with different amounts of food and noting which individuals reach the pupal stage. This has been done by CHIANG & HODSON (1950), using *Drosophila melanogaster* as experimental animal. In another experiment they limited the time during which food was available to the larvae. From a large group of larvae which was reared on sufficient food, they took samples every twelve hours and transferred them to rearing chambers without food. The number and size of the pupae that appeared in each group were recorded. The results of these two experiments could be combined on the basis of the wing length of emerging adults, which was used as a measure for the size of the animal. In this way the feeding period could be connected with quantity of food consumed, which gave an estimate of the rate of food consumption.

BEADLE, TATUM & CLANCY (1938) have described an experiment which is very similar to the second experiment of CHIANG & HODSON mentioned above. They studied the minimum feeding period necessary for the larvae of *D. melanogaster* to be able to pupate, the weight of adults which had fed for different periods during their larval stage, and the influence of different periods of starvation and subsequent feeding on the moment of pupation. Their results may be summarized as follows:

1. Larvae which had fed less than 48 hours at 25° C did not pupate, while those that fed 48 hours or longer pupated. The minimum period of feeding coincides with the period necessary to reach the third instar.
2. Larvae which were deprived of food for different periods before passing into the third instar were retarded in development. The retardation was a function of the length of the starvation period.
3. If the larvae were deprived of food after the second moult, no retardation in development was noted. Probably the development was even somewhat accelerated.
4. If the larvae were deprived of food after the second moult for different periods, the final weight of the emerging adults was dependent on the length of the period spent on full food.

Though the main purpose of the investigations of ALPATOV (1930) was to study the relation between body and cell size, some interesting data relating to the present subject can also be found in his paper. One of his experiments concerning variation in body size consisted of the removal of larvae from the food after different periods. The shortest feeding period tested by him was 48 hours at 25° C. Of 151 larvae which had fed for 48 hours, only 30 adults emerged, i.e., 20%. With longer feeding periods, this percentage increased: e.g. 52 hours: 47%, 56 hours: 57%, 60 hours: 78%, 64 hours: 95%.

The sex ratio of the strain used was 86% ♂♂ (in percentage of ♀♀). Males always pupated earlier than females. Larvae with subnormal feeding periods appeared to pupate earlier than larvae with normal feeding periods. As Alpatov found 103% ♂♂ emerging from underfed larvae (48—80 hrs of feeding), he concluded that males might be more resistant to underfeeding.

The present investigation is a further elaboration of the experiments of BEADLE, TATUM & CLANCY (1938) and CHIANG & HODSON (1950). Its point of view may be called ecological. The main underlying problem can be stated as follows: How do different degrees of underfeeding affect the chances of the larvae to survive ? It does not deal with the physiology of the endocrine control of development, though this subject is intimately interwoven with the present problem, as inanition seems to affect primarily neurosecretion (JOHANSSON, 1958). In this way, the ultimate effects of inanition are cessation of growth, puparium formation, and, in adult insects, reproduction.

MATERIAL AND METHODS

The animals used belonged to a strain of *D. melanogaster* which was obtained by intercrossing four different wild strains. The strain was reared at 25° C and at constant densities in jars containing a constant amount of food. A system of cyclic matings has been maintained in order to reduce inbreeding. The viability of the strain is very good. At the time of the experiment the strain had been kept for 21 generations in this laboratory.

The medium used was the agar medium of KALMUS (1943) of which the sugar was omitted, in order to prevent yeast growth. It consists of:

agar	20	grams
tartaric acid	5	,,
KH_2PO_4	1.5	,,
$(NH_4)_2SO_4$	2	,,
$MgSO_4 . 7H_2O$	0.5	,,
water	971	,,
nipagine 10% in alcohol 96%	10	ml

The salt mixture should be added to the agar solution below 70° C to avoid hydrolysis of the agar.

Jars of 35 mm high and with a diameter of 55 mm, and jars of 80 mm high and with a diameter of 45 mm were filled with 15 and 10 cc of this medium respectively. The two different sizes of jars were chosen, because the larvae could be picked up more easily out of the lower ones, whereas the walls of the higher ones could be used as pupation sites for larvae which were ready to pupate.

The food for the larvae was prepared by suspending 20 grams of "Engedura", a living dried yeast manufactured by the "Koninklijke Nederlandse Gist-en Spiritusfabrieken" at Delft, in 100 ml suspension. One ml of this suspension was added to each jar.

As we wanted to have data on increase in weight at intervals of 6 hours, it appeared more convenient to use two groups of larvae, differing twelve hours in age. In this way it could be arranged that the most laborious part of the work was done during the day, while we still could sample with 6 hour intervals as regards age of larvae.

Females of 7—8 days old were allowed to oviposit on watch glasses containing ca 3 ml of the agar medium with a drop of a thick suspension of yeast added in the middle.

In the jars in which the parents were kept, large numbers of larvae crawled around. Oviposition is much lowered under such conditions. The females will retain their eggs, though one of these eggs in each female may be fertilized and may develop for some time in the uterus. When this egg is eventually laid, it will not be at the same stage of development as the others which are laid immediately afterwards. Therefore, the eggs laid during the first half hour of each oviposition period were not used in the experiment. Oviposition periods were:

1. From 10.00 to 10.30 and from 10.30 to 12.30.
2. From 22.00 tot 22.30 and from 22.30 to 0.30.

The yeast was washed off the oviposition glasses and these were put into small petri-dishes in the incubator; about twenty-three hours later, between 9.15 and 10.15, and between 21.15 and 22.15 respectively, 50 newly hatched larvae were transferred to each culture jar. For every 6-hour check two jars were prepared, A and B. On following days observations were made at 9.30 and 15.30. Both times 2 sets of 2 jars A and B were taken out of the incubator, containing animals that differed twelve hours in age. The animals from the jars "A" were picked out, washed with tap water and counted, and their larval stage was ascertained. Then they were transferred to new jars (A') with 10 ml of agar medium but without any food. The animals from the jars "B" were treated in the same way, but instead of a transfer to new jars they were weighed together alive. Afterwards they were dried at 110° C for about 12 hours and weighed again. It appeared very convenient to place the animals on small strips of aluminium foil, which could be made of an adequate size.

From an age of 84 hours after hatching all jars A' were examined every 6 hours to record the moment of puparium formation. In addition, the jars A with larvae and/or pupae that were not yet transferred, were examined for the same purpose.

The emerging adults were collected at intervals of about 12 hours; their sex was determined and they were weighed twice: alive and dried at 110° C. The pupal cases found in the jars A' were collected and weighed also.

RESULTS

Duration of different stages

From Table I it can be seen that the first instar is completed in about 24 hours after hatching and transfer to the food. The second molt occurs between 42 and 48 hours, as no second instar larvae are found after 48 hours. Puparium formation in animals that spent their entire larval period on the food takes place in this strain at about 90 hours after hatching. These findings show general agreement with the figures given in DEMEREC (1950), though puparium formation appears to be slightly earlier here.

The moment of puparium formation in larvae that had passed the second moult was influenced by the duration of the feeding period only in larvae which were taken from the food after 48 hours of feeding. These larvae were somewhat retarded in development. Table II shows that 96 hours after hatching more than 80% had finished puparium formation in all series except the first of 48 hours. At 102 hours after hatching, in this series only 41% had formed a puparium, whereas in larvae which had stayed longer on the food, this percentage was more than 95%. This is not in agreement with the opinions of BEADLE, TATUM & CLANCY (1938) who thought they found a slight acceleration of development in larvae in which the feeding period was broken off after the second moult.

It is also at variance with the findings of ALPATOV (1930) who found that larvae with a feeding period of 48 hours pupated on the average 13 hours earlier than normally fed larvae. He stated, however, that in another experiment, conducted under the same conditions, underfed larvae were on the average much less accelerated as compared to normally fed ones. In similar experiments on *Calliphora erythrocephala*, EZHIKOV (1917) and COUSIN (1926) found a distinct acceleration of development by shortening of the larval period when larvae are deprived of food. The reduction of the larval period was dependent upon the duration of the feeding period.

TABLE I

Duration of different developmental stages and fate of larvae that were transferred to jars without food after different periods.

period on food (hours) = age	Stage of development at the moment of transfer					number of puparia formed	number of larvae found	number of larvae escaped	% pupation of remaining larvae	adults emerging			
	eggs	I	II	III	pupae					♂♂	♀♀	total	% of puparia
—23	50	—	—	—	—								
0	—	50	—	—	—								
12	—	42	—	—	—								
18	—	42	—	—	—								
24	—	14	24	—	—								
30	—	1	42	—	—								
36	—	—	46	—	—								
42	—	—	42	—	—								
48	—	—	—	44	—	22	1	21	96	11	7	18	82
54	—	—	—	45	—	45	—	—	100	18	20	38	84
60	—	—	1	34	—	29	—	6	100	18	10	28	97
66	—	—	—	49	—	43	—	6	100	23	19	42	98
72	—	—	3	37	—	36	—	4	100	16	20	36	100
78	—	—	—	49	—	47	—	2	100	22	20	42 *	91
84	—	—	—	47	—	36	—	11	100	18	14	32 *	91
90	—	—	—	25	20	44	—	1	100	24	17	41	93
96	—	—	—	2	47	48	—	1	100	20	28	48	100
102	—	—	—	—	44	44	—	—	100	19	25	44	100
108	—	—	—	—	48	48	—	—	100	23	24	47	98
114	—	—	—	1	48	48	1	—	98	27	21	48	100
120	—	—	—	1	47	48	—	—	100	17	30	47	98
144	—	—	—	—	48	48	—	—	100	25	23	48	100
168	—	—	—	—	48	48	—	—	100	26	19	45	94

* 1 puparium damaged.

Minimal feeding period

Only larvae that had stayed on the food for 48 hours or longer were able to pupate (Table I). The number of puparia found, however, does not correspond with the number of larvae transferred. E.g., of 44 third instar larvae transferred after 48 hours, 22 pupae were formed. Only 1 larva could be recovered so it is assumed that 21 escaped from the jars. The jars were closed with screw tops, which apparently were not larvae-tight. This assumption is not unfounded as will be clear from the following arguments. It might be supposed that the remaining larvae had reached the pupal stage by means of cannibalism on their brethren. In this case the mouthparts of the missing larvae would have been retrieved, as they are clearly discernable in the agar. They were, however, not found. Moreover, larvae with a feeding period of 54 hours all pupated, whereas from later series various numbers are missing. Further it is known that larvae of *D. melanogaster* (ALPATOV, 1930) and *C. erythrocephala* (COUSIN, 1926) are very active after they are taken from the food. Finally, some larvae have actually been found between the screw top and the glass wall.

However, of the remaining 23 larvae, 22 pupated and it can therefore be reasonably supposed that the missing larvae would have pupated in largely the same proportion, i.e. 96%.

The great influence of the feeding period on the percentage of larvae which are able to pupate, found by ALPATOV (1930) and CHIANG & HODSON (1950) could not be demonstrated here. In *Calliphora erythrocephala,* COUSIN (1926) found nearly 100% pupation, even with the shortest feeding period.

Complete agreement is thus found with the results of BEADLE, TATUM & CLANCY (1938) mentioned in the introduction. The results of the experiment of CHIANG & HODSON (1950) are not wholly comparable, as it was conducted at room temperature instead of at 25° C. The possibility of the larvae to pupate after only feeding a relatively short time has very important biological effects as it secures further development and survival when food uptake is broken off early. The larvae, however, are not accelerated in development: if the food supply is restored and conditions allow them to resume feeding, growth is continued to the benefit of future reproduction. If the larvae have passed the second moult, no retardation in development is observed either as a consequence of the interruption in food uptake, except in larvae which had fed only 48 hours. Puparium formation is thus not postponed when conditions enable the larvae to feed for 54 hours or longer. In this way loss of weight as a consequence of starvation is restricted.

The endocrine mechanism is apparently not influenced by inanition after 48 hours of feeding in such a way as to prevent puparium formation. However, further development is not always secured when puparium formation has taken place, as in overcrowded cultures often many small puparia can be found which apparently have not developed further. Such puparia were very rarely seen in this investigation. Since the intervals between two controls, however, were 6 hours, it is possible that inanition after some period between 42 and 48 hours will result in puparium formation without further development.

The "période d'alimentation indispensable" (BOUNHIOL, 1938) is thus about

TABLE II

Moment of puparium formation of larvae which stayed on the food for different periods. In every vertical column the observations below the line are made before transfer.

age in hours	84		90		96		102		108		114		126		132		144	
hours before transfer	number	%	number	%	number	%	number	%	number	%	number	%	number	%	number	%	number	%
48					6	27	9	41	16	73	18	82	19	86	?	?	22	100
54			23	51	42	93	45	100										
60	2	7	20	69	24	83	28	97	?	?	29	100						
66			32	74	42	98	43	100										
72			7	19	36	100												
78			3	6	47	100												
84			9	25	31	86	36	100										
90			20	45	41	93	42	95	?	?	43	98	?	?	44	100		
96			14	29	47	98	47	98	48	100								
102			12	28	42	98	43	100										
108			?	?	44	92	?	?	48	100								
114			?	?	46	96	?	?	?	?	48	100						
120			26	55	44	94	47	100										
144			25	52	48	100												
168			?	?	48	100												

48 hours for *D. melanogaster.* The end of this period does not seem to coincide with the end of the "critical period" during which the pupation hormone is released. This critical period can be found when ligatures are made on different moments before puparium formation. For *D. hydei* (VOGT, 1942) the critical period lies about 12—4 hours before puparium formation, i.e., when the anterior part of the larva was separated from the posterior part by means of a ligature, the posterior part was prevented from pupating if the ligature was made earlier than 12 hours before puparium formation. In *D. melanogaster* this critical period does certainly not end already about 48 hours before puparium formation, i.e., on the moment when the larvae which are put into inanition have passed the second moult and will succeed to form a puparium. No acceleration of puparium formation as a consequence of inanition has been observed, so, during the starvation period the endocrine system depends entirely on the quantity of food ingested during the first 48 hours. No doubt, due to the entirely different technique, the results of these two types of experiments are not directly comparable.

Pupal mortality

Though my data are insufficient for far reaching conclusions, it is quite obvious that pupal mortality is rather small, being above 10% only in case of larvae which had fed for 48 or 54 hours (Table I).

Sex ratio

No indication could be found of an influence of feeding period on the sex ratio of emerging adults (Table I, Fig. 1). The ratio's appear scattered around 50%. The preponderance of males emerging from underfed larvae, found by ALPATOV (1930) for *D. melanogaster* and by HERMS (1928) for *Lucilia sericata* was not found in our strain. ALPATOV founded his conclusion upon the difference between the percentage of males (♂♂ in percentage of ♀♀) in normally fed larvae and underfed larvae, 86 and 103% respectively. His figures are 356 ♂♂ vs. 415 ♀♀ in normally fed larvae, and 513 ♂♂ vs. 500 ♀♀ in underfed

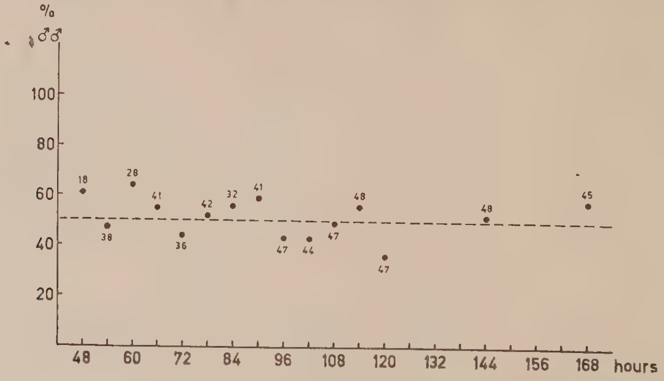

Fig. 1. Sex ratio of adults emerging from pupae formed by larvae with different feeding periods. (Abscissa. feeding period; ordinate: percentage of males).

larvae. For normally fed larvae, the χ^2 test gives a value for χ^2, which is significant at the 5% level. On the basis of ALPATOV's figures, the sex ratio of his strain was 4.6 ♂♂ : 5.4 ♀♀. For underfed larvae, χ^2 becomes 8.7, which is significant at the 1% level. The figures given by HERMS for *L. sericata* are not very convincing; only in one out of four cases in which underfeeding caused considerable mortality, χ^2 was significant at the 5% level. Larvae transferred after 84 hours of feeding were predominantly females, which was obviously caused by earlier pupation of the males.

Larval growth and pupal weight

All observations concerning the weighings are embodied in Table III and Figures 2, 3 and 4. In considering the results, it should be kept in mind that all data are derived from different sets of larvae. Comparison of data is therefore only allowed, if it can be assumed that variability in the different sets of 50 larvae is largely the same. We have no reason to believe that this is not the case.

TABLE III

Mean live and dry weights in mg, and percentage water content of eggs, larvae, and puparia on different moments.

	eggs and larvae					puparia			
Period on food (hours) = age	number	stage	mean live weight	mean dry weight	% water content	number	mean live weight	mean dry weight	% water content
—23	50	egg	0.0098	0.0022	77.6				
0	50	I	0.017	0.0022	87.1				
12	32	I	0.026	0.0056	78.5				
18	32	I	0.049	0.0105	78.6				
24	29	II	0.063	0.0173	72.6				
30	36	II	0.177	0.0334	81.2				
36	35	II	0.312	0.0537	82.8				
42	37	II	0.476	0.0873	81.7				
48	33	III	0.812	0.1476	81.9				
54	33	III	1.303	0.2321	82.2				
60	38	III	1.652	0.3111	81.2				
66	29	III	1.958	0.4110	79.1				
72	40	III	2.280	0.4788	79.0				
78	35	III	2.068	0.5020	75.8	1	1.870	0.620	66.8
84	50	III	2.000	0.5336	73.3				
90	9	III	1.909	0.5111	73.3	35	1.634	0.506	69.1
96	4	III	1.930	0.5075	73.7	45	1.600	0.507	68.3
102						48	1.397	0.485	65.3
108						41	1.371	0.488	64.5
114						47	1.391	0.494	64.5
120						48	1.339	0.487	63.7
144						44	1.364	0.482	64.7
168						48	1.465	0.463	68.4

Figures 2 and 3 give the course of live weight and dry weight of larvae and

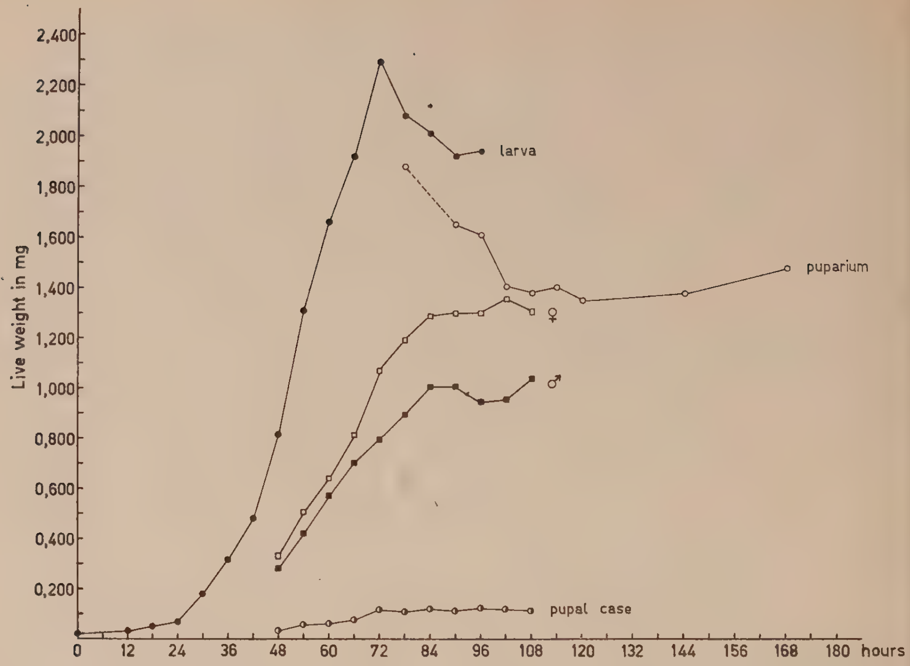

Fig. 2. Live weight of larvae and puparia of different ages; live weight of ♂♂ and ♀♀ originating from larvae with different feeding periods. Weights of pupal cases of these adults.

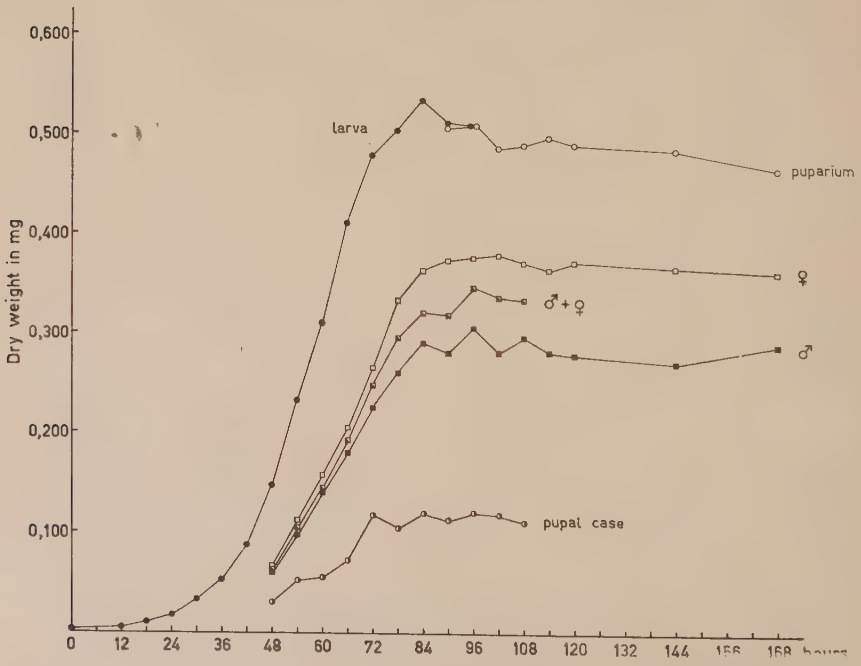

Fig. 3. Dry weight of larvae and puparia of different ages; dry weight of ♂♂ and ♀♀ (separate and together) originating from larvae with different feeding periods, and weights of pupal cases of these adults.

puparia. These graphs should be consulted together. After 72 hours of feeding, live weight decreased. As a further increase in dry weight is observed up to 84 hours (Fig. 3), the decrease in live weight must be caused by water loss. Water loss is again observed at puparium formation and during the first twelve hours of the pupal stage. The slight decrease in dry weight of the puparium may perhaps be attributed to metabolic processes during metamorphosis.

When increase in weight is plotted logarithmically against time (Fig. 4), it can clearly be seen that the coefficient of increase, $c = \frac{1}{gt} \cdot \frac{dg}{dt}$, the increase per unit of time with regard to the weight g at the moment t, remains the same up to 54 hours. Then this coefficient decreases, becoming zero at 84 hours.

Weight of adults with different larval feeding periods

In Table IV and in Figures 2, 3 and 4 mean weight of adults are given which had emerged from the puparia formed by larvae with different feeding periods. It is interesting to note that the sex difference in weight, which is very pronounced in animals that did not suffer from food shortage, can already been found in adults emerging from larvae that had fed only 48 hours. Rate of food consumption and/or efficiency in use made of the food consumed — i.e. the proportion of the food consumed which is ultimately incorporated in the adult body — must therefore be higher in the female larva.

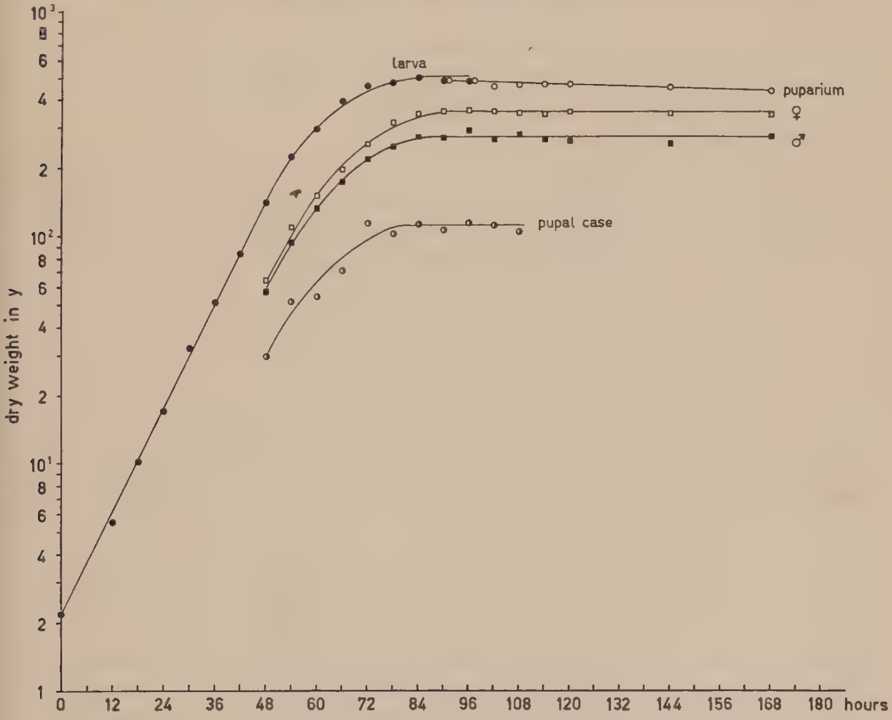

Fig. 4. Same as figure 3, but weights on logarithmic scale.

TABLE IV

Weights of adults emerged from puparia formed by larvae with different feeding periods and weights of their pupal cases (in mg). Percentage weight loss during starvation period.

age before transfer	♂♂	mean live weight	mean dry weight	% water	♀♀	mean live weight	mean dry weight	% water	mean dry weight ♂♂ + ♀♀	mean weight pupal case	mean weight adults + pupal case	mean dry weight larva	weight difference	% weight loss	mean dry weight puparium	weight difference	% weight loss
48	11	0.277	0.059	79.0	7	0.329	0.066	80.0	0.062	0.031	0.093	0.148	0.055	37.2	—	—	—
54	18	0.419	0.098	76.6	20	0.506	0.113	77.7	0.106	0.053	0.159	0.232	0.073	31.5	—	—	—
60	18	0.569	0.139	75.6	10	0.639	0.157	75.5	0.146	0.056	0.202	0.311	0.109	35.0	—	—	—
66	23	0.700	0.180	74.3	19	0.809	0.206	74.6	0.192	0.073	0.265	0.411	0.146	35.5	—	—	—
72	16	0.791	0.225	71.6	20	1.067	0.266	75.0	0.247	0.118	0.365	0.479	0.114	23.7	—	—	—
78	22	0.889	0.259	70.9	20	1.185	0.333	71.9	0.294	0.106	0.400	0.502	0.102	20.3	—	—	—
84	18	1.000	0.289	71.1	14	1.278	0.363	71.6	0.321	0.119	0.440	0.534	0.094	17.4	—	—	—
90	24	1.000	0.281	71.9	17	1.291	0.372	71.2	0.318	0.112	0.430	0.511	0.081	15.9	0.506	0.076	15.0
96	20	0.941	0.306	67.5	27	1.290	0.375	71.0	0.345	0.120	0.465	0.508	0.043	8.5	0.507	0.042	8.3
102	19	0.952	0.281	70.5	25	1.348	0.377	72.1	0.336	0.117	—	—	—	—	0.485	0.032	6.5
108	23	1.030	0.296	71.3	23	1.293	0.369	71.5	0.332	0.109	—	—	—	—	0.488	0.047	9.7
114	27	—	0.279	—	21	—	0.362	—	—	—	—	—	—	—	—	—	—
120	16	—	0.278	—	30	—	0.371	—	—	—	—	—	—	—	—	—	—
144	25	—	0.269	—	23	—	0.364	—	—	—	—	—	—	—	—	—	—
168	25	—	0.287	—	19	—	0.359	—	—	—	—	—	—	—	—	—	—

The puparia were not weighed, nor kept apart, so it could not be found out whether the males stored relatively more material in their pupal case, as is demonstrated in *Musca domestica* (FUCHS, in litt.).

Mean weight of pupal cases was ascertained and added to the mean weight of the adults. For this purpose, weights of males and females were taken together, because the sex of the pupae was unknown. Figures obtained give mean dry weight of puparia. These could be compared with mean dry weight of larvae. It is clear that larvae, removed from the food at different moments after the second moult will loose weight as a consequence of metabolism. This loss of weight will be dependent upon the duration of the starvation period between the removal from the food and puparium formation. In Fig. 5 this is clearly demonstrated. The percentage loss of dry weight of larvae (and puparia) which stayed on the food during the period indicated on the abscissa is calculated as follows:

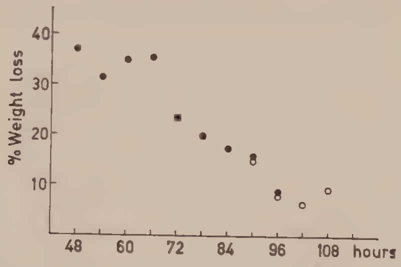

Fig. 5. Percentage of weight loss during starvation period and pupal stage. For calculation see text. ● larvae, ○ puparia. Abscissa: Age of animals at the moment of transfer to the agar without food.

$$\frac{100\ (\text{mean weight of larva or puparium}) - (\text{mean weight of adult} + \text{mean weight of pupal case})}{\text{mean weight of larva or puparium.}}$$

According to these results, up to 38% of the weight acquired during the feeding period may be lost.

From the graph it can be seen that loss of weight in the course of the pupal stage is about 6—15%.

Water content during development

As both live and dry weight of larvae, puparia and adults were measured, the water content could be easily determined. Figure 6 gives the results. The fluctuations in percentage water found during the first 30 hours are probably due to experimental errors. The water content is calculated as a quotient and small differences in live or dry weight which are due to weighing errors, give rise to great differences in percentage water content. However, with increasing weight, this source of errors decreases. Between 30 and 54 hours the water content remains fairly constant at about 82%, but afterwards there is a clear drop to about 73% at 84 hours. Puparia, again, have a lower percentage of water. It varies between 69 and 63%, being somewhat higher at the beginning of the pupal stage. The observation at 168 hours lies outside the general trend observed.

Adults coming from larvae with different feeding periods differed in water content. This was higher in adults with shorter feeding periods during their larval stage. The same phenomenon has been found by Fuchs (*in litt.*) in *Musca domestica.* A small sex difference in this respect may also noted: females have a somewhat higher water content.

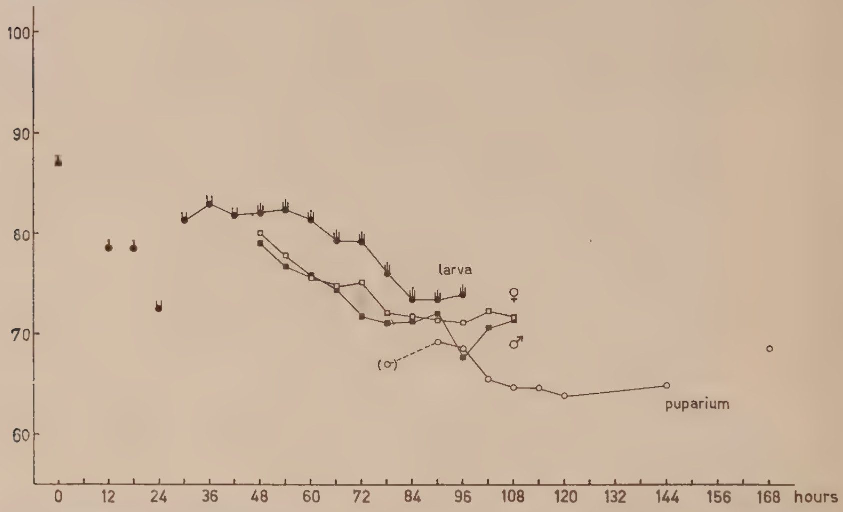

Fig. 6. Percentage of water of larvae and puparia of different age. Percentage of water of ♂♂ and ♀♀ ultimately originating from larvae with feeding periods which are marked on the abscissa.

| ● larvae 1st instar
|| ● ,, 2nd ,,
||| ● ,, 3rd ,,

Length of larvae

Alpatov (1929) in his work on the growth of *Drosophila melanogaster* larvae has measured length. For this purpose, larvae of different ages were killed in boiling water which has the advantage that the larvae are stretched very neatly. He preserved the perfectly straight larvae obtained in alcohol 70% before examination. Measurements were taken by means of an ocular micrometer in a microscope or binocular microscope.

In the present investigation samples of larvae from the B-jars were treated in the same way, except for the fact that they were not put in alcohol but were measured immediately in water after being killed. The results were very similar to those of Alpatov. He found during each instar a gradual decrease in growth rate at the end of each larval stage. At the subsequent moults, larval length suddenly increases. Comparison of his data with Fig. 3 (or 4) shows, that there are no irregularities in the increase of weight. The only explanation for this seeming contradiction is a changing ratio between length and diameter of the larvae during each instar.

Further speculation seems, however, premature as we do not know which changes are brought about by the fixation technique.

I wish to thank Prof. Dr. D. J. KUENEN and Prof. Dr. J. DE WILDE for their critical remarks on the manuscript and Mr. J. W. DE MEIJER for his careful assistance during this investigation. I owe much to inspiring discussions with Mr. P. FUCHS on our subject of mutual interest.

ZUSAMMENFASSUNG

FRAßDAUER, WACHSTUM UND VERPUPPUNG BEI LARVEN VON DROSOPHILA MELANOGASTER

1. Ein Experiment wird beschrieben, bei dem die Nahrungsaufnahme von *Drosophila*-Larven nach verschiedenen Zeiträumen unterbrochen wurde. Die Larven wurden teils auf einen Agarboden ohne Nahrung überführt und teils gewogen, getrocknet und wieder gewogen.

2. Die Dauer der verschiedenen Entwicklungsstadien war bei ungestörten Larven ungefähr dieselbe, wie sie DEMEREC (1950) angibt. Nur der Beginn der Puparienbildung lag etwas früher. Wenn die Larven nach der zweiten Häutung auf den Agarboden ohne Nahrung überführt wurden, gingen Larven, welche nur 48 Stunden gefressen hatten, etwas später zur Puparienbildung über. Die Entwicklung war nicht verzögert bei Larven, welche länger gefressen hatten.

3. Nur Larven, welche mindestens 48 Stunden gefressen hatten, verpuppten sich. Sie müssen also die zweite Häutung vollzogen haben. Es wurde keine größere prozentuelle Zunahme von Verpuppungen gefunden, wenn die Larven länger gefressen hatten. Die Mortalität der Puppen war etwas größer, wenn die Tiere nur 48 oder 54 Stunden fressen konnten.

4. Das Geschlechterverhältnis der schlüpfenden Imagines wurde von der Dauer des Aufenthalts auf dem Nährboden nicht beeinflußt.

5. Der Gewichtszunahmekoeffizient der Larven, $c = \frac{1}{gt} \cdot \frac{dg}{dt}$, blieb bis 54 Stunden derselbe. Danach nahm er ab und wurde ach 84 Stunden null.

6. Das Lebendgewicht der Larven war nach 72 Stunden am höchsten, während das Trockengewicht noch bis zu 84 Stunden zunahm. Dieser Unterschied wurde durch den Wasserverlust der Larven verursacht, welcher der Puparienbildung vorausgeht. Während der Puparienbildung und der ersten 12 Stunden des Puppenstadiums findet weiterer Wasserverlust statt. Die kleine Abnahme des Trockengewichts während des Puppenstadiums wird vielleicht durch metabolische Prozesse verursacht.

7. Das Gewicht der Imagines war stark von der Zeitdauer abhängig, während der die Larven fressen konnten. Der Gewichtsunterschied zwischen den Geschlechtern trat schon bei Larven deutlich in Erscheinung, die nur 48 Stunden gefüttert wurden. Bei den weiblichen Larven muß die Nahrungsaufnahme also schneller vor sich gehen und/oder der Nutzeffekt der aufgenommenen Nahrung größer sein.

8. Während der Hungerperiode zwischen der Überführung auf den Agarboden und der Puparienbildung fand ein Gewichtsverlust statt, der um so größer war, je länger die Hungerperiode dauerte.

9. Der Wassergehalt zeigte während des dritten Stadiums eine deutliche Abnahme. Während des Puppenstadiums betrug er nach den ersten 12 Stunden ziemlich konstant 64%. Der Wassergehalt der Imagines, die von Larven mit verschiedenen Nahrungsaufnahmezeiten stammten, erwies sich von der Dauer dieser Fraßperioden abhängig.

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THE ANNUAL CYCLE OF *LIMOTHRIPS CEREALIUM* HALIDAY (THYSANOPTERA) AND ITS DISTRIBUTION IN A WHEAT FIELD

BY

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Terrestrial and aerial populations of *L. cerealium* were studied simultaneously; ovarian dissections were made to detect different generations. The annual life cycle can be arbitrarily divided into six stages based primarily on the amount of flight activity: the females feed mainly on winter cereals and grasses in spring, many moving to later sown cereals in June and July, where most eggs are laid in the sheaths of the young leaves. First generation females leave cereals in August to feed on grasses before entering hibernation.

Wheat ears with a water content of less than approx. 45% were not favoured by thrips. When winds blew consistently from the same quarter the initial infestation was greatest on the windward edge of the wheat crop.

Adult female *Limothrips cerealium* fertilised the previous summer, hibernate in plant debris and under bark (PRIESNER, 1925; MORISON, 1943). In spring they appear on grasses and young cereals, which are often heavily infested by early summer. They feed and oviposit on the leaf bases and in the leaf sheaths at first, moving towards more tender parts as the leaves become tough. SHARGA (1933) described oviposition and hatching. On cereals larvae from the earlier laid eggs feed and shelter in the leaf sheaths and developing ears. When the ears emerge they may move inside the glumes, where later eggs are laid, and then on to the young ovaries. Pupation takes place on the plant. By late June or early July the apterous males appear; and adults are most abundant in late summer (MORISON, 1928; KÖRTING, 1930). Males die after mating (SHARGA, 1933) but females leave the host plants for winter quarters in August and September, sometimes in large "swarms". (KÖRTING, 1930; VON OETTINGEN, 1942).

Two generations per year have been recorded in N.E. Scotland (MORISON, 1943) and in N. Germany (KÖRTING, 1930). In Sweden, JOHANSSON (1938) considered that a partial third generation may sometimes develop on grasses. SHARGA (1933) recorded only one generation in E. Scotland; he gives the life history under "laboratory conditions" as follows:— egg 10—13 days; 1st instar 5—7, 2nd instar 8—10; pre-pupa and pupa 2—3 and 6—7 days respectively, a total of 20—35 days. VAN EECKE (1922) estimated that it took at least 35 days, and KÖRTING (1930) found it was 26—28 days at 18.5° C.

All cereals are susceptable to infestation; KÖRTING (1930) gives the order of preference as wheat, rye, barley, oats. MORISON (1943) found females on 29 species of Gramineae and on 48 other species of flowering plants; males were restricted to certain *Gramineae* and plants adjacent to them in the field.

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The aim of the work was to study the annual cycle of *L. cerealium* at Silwood Park, and the distribution and development of a thrips infestation on and around a cereal crop; terrestrial and aerial populations of *L. cerealium* were studied simultaneously in 1956, 1957 and 1958.

METHODS

Experimental Site

Silwood Park is situated in a well wooded district near Windsor Great Park; throughout the work only a small acreage of cereals, mainly wheat and barley were grown within 3 miles.

The experimental site was a 3-acre field, roughly trapezoidal in shape, lying in a small valley running S.W. to N.E. The soil, a sandy-loam overlying a hard pan, was often waterlogged in winter but quickly dried out in summer. The N.W. boundary adjoined a dense, deciduous copse and to N.E. and S.E. areas of waste grassland separated the field from woods 50 to 200 yards away. On the S.W. edge a strip of rough grassland, about 30 yds wide, separated the wheat from a 5-acre field of spring oats. To the S.W. the valley bottom was treeless for about 500 yds.

Spring wheat (var. Atle) was grown in all seasons. Along the S.W. and S.E. edges of the field, scattered wheat plants, self set from seed dropped at harvest, appeared late in 1956 and 1957. They were allowed to grow, and sampled in the following seasons as "winter" wheat. In 1957, duplicate plots of winter oats (S. 147) and timothy (*Phleum pratense*) were grown along the S.W. edge of the field, and of cocksfoot (*Dactylis glomerata*) and perennial rye grass (*Lolium perrenne*) along the S.E. edge. Each plot measured 5 × 10 yd. The winter oats, severely attacked by powdery mildew (*Erysiphe graminis* DC.) and birds, were unfit for sampling after mid July; spring oats were subsequently sampled at 2 sites of similar size in the adjoining field. The oats and grass plots were grown again in 1958.

Vegetation Sampling

Sampling sites, each 10 × 10 feet, were selected in the wheat crop; 9 sites were sampled in 1956 (Fig. 1a) and 7 in 1957 (Fig. 1b). One sample of 15 tillers was taken from each oats or grass plot on each occasion. Tillers were cut at ground level until the wheat reached growth stage 10.5 (LARGE, 1954) or, in the case of grasses, until the stalks withered; thereafter only the ears or inflorescences were taken. Thrips were extracted with turpentine (LEWIS, 1960). The number of *L. cerealium* obtained from all samples of each "host" gave an estimate of its relative abundance on that "host" throughout the season. The coefficient of variation of the complete sampling method determined during May and June was ± 11% and ± 20.4% in 1956 and 1957 respectively. The size of samples, and the sampling dates, are given in Table I.

TABLE I

Hosts sampled in 1956 and 1957

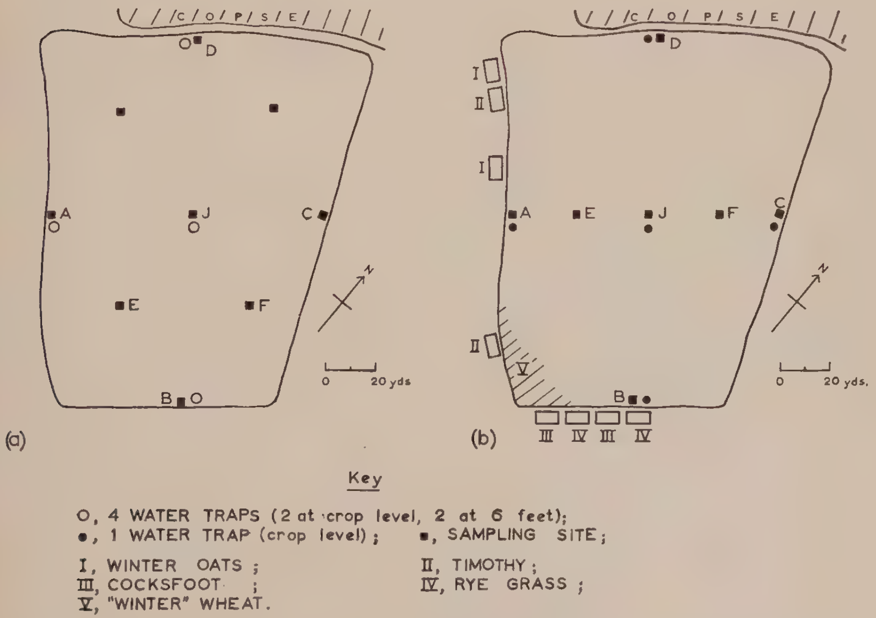
Host	Sampling Started	Sampling Finished	Sampling Interval (days)*	Total tillers per sampling occasion
Spring wheat, 1956	9/5	11/9	2	90
Spring wheat, 1957	22/5	25/8	5	105
"Winter" wheat ,,	7/5	26/7	5	30
Winter oats ,,	20/4	28/7	5	30
Spring oats ,,	14/7	24/8	5	30
Rye grass ,,	21/4	9/9	5	30
Cocksfoot ,,	21/4	29/8	5	30
Timothy ,,	20/4	9/9	5	30

* Except in wet weather when sampling was postponed a day.

No set sampling programme was followed in 1958.

Trapping

White, water traps were used to detect flight activity (LEWIS, 1959). In 1956, two traps at crop level and two at 6 feet were placed at each of the sites A, B, D and J (Fig. 1a), in 1957 there was one trap at crop level at sites A, B. C, D and J (Fig. 1b). Trapping extended from 23rd April—27th September, 1956 and from 14th April—26th August, 1957. The total daily catch in the 16 traps of 1956 and the 5 of 1957 was used as an indication of daily flight activity.


C O P S E
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(a)
C O P S E
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0
20 yds.
III IV III IV
(b)
Key
O, 4 WATER TRAPS (2 at crop level, 2 at 6 feet);
●, 1 WATER TRAP (crop level); ■, SAMPLING SITE;
I, WINTER OATS; II, TIMOTHY;
III, COCKSFOOT; IV, RYE GRASS;
V, "WINTER" WHEAT.

Fig. 1. Sampling and trapping sites in the spring wheat crop. (a) 1956, (b) 1957.

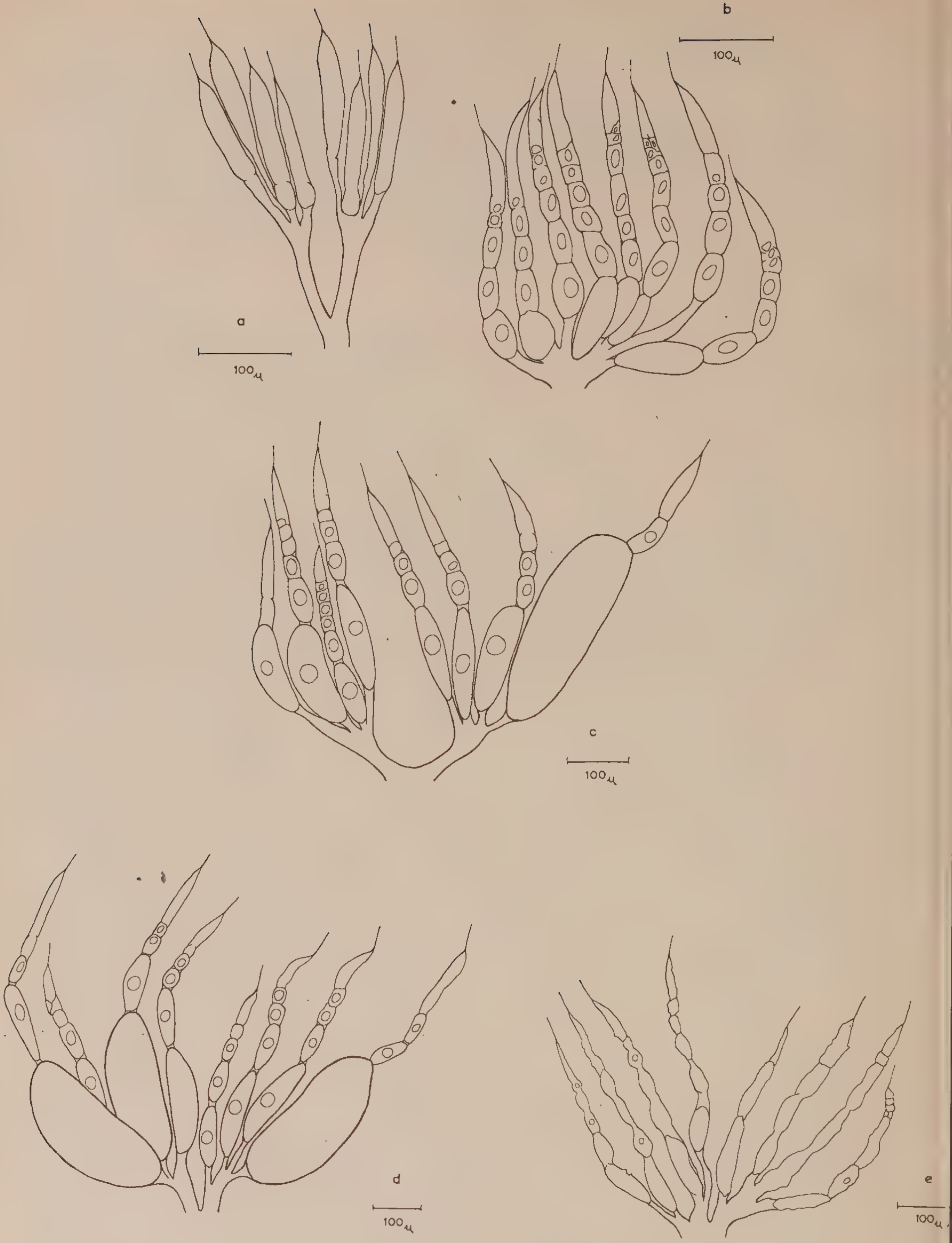

Fig. 2. Ovaries of *L. cerealium* showing stages in development.
(a) Undifferentiated or "Immature".
(b) Early maturing.
(c) Late maturing.
(d) Laying.
(e) Senile

Dissection of Ovaries

Ovaries, of females taken from plants or in flight, were examined at intervals throughout 1957 to determine the number of generations per season. Thrips were dissected as soon as possible after collection; fresh ones gave best results and dissections were difficult if the insects had remained for more than a day in the water traps. Thrips were dissected on a slide under a binocular microscope. The anterior of the abdomen was pressed against the slide with a needle and the ovaries removed by pulling the last two abdominal segments with forceps. Dissections were mounted in Goto's modification of polyvinyl lactophenol[1]), slightly coloured with lignin pink.

Ovaries were classified into four categories, thus: (i) Undifferentiated and Immature — longest egg rudiment $< 50\mu$, (ii) Maturing — longest egg or rudiment 50—350 μ, (iii) Laying — longest egg $>$ 350 μ, (iv) Senile — shrivelled ovaries. See also Fig. 2.

In borderline cases the state of all ovarioles was considered, as well as the measurements. Each estimate of the percentage of females in each category was based on 30—40 dissections.

RESULTS AND DISCUSSION

THE ANNUAL CYCLE OF *L. CEREALIUM*

Numbers taken from vegetation and from the air are plotted in Figs. 3, 4 & 5. The graphs are not exactly comparable for different seasons or hosts, because sample sizes and the number of traps used were different, but they show the relative fluctuations in numbers of larvae and adults on different hosts and in the air throughout the season.

All thrips larvae were plotted[2]); on wheat about 98% were *L. cerealium*, and on winter and spring oats the proportions of *L. cerealium* were 30% and 90% respectively; most of the others were *Stenothrips graminum* Uzel.

The annual cycle can be arbitrarily divided into six stages, based primarily on the amount of flight activity (Fig. 3a, 4a).

Stage 1: Emergence from Winter Quarters. Movement from winter quarters resulted in a period of flight activity in spring. In 1956, there was a distinct peak of activity during the first half of May, but in 1957 this peak was hardly detecta-

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[1]) Goto's modification: —

5 g .... polyvinyl alcohol (Bush, Beach and Gent. Beacon Brand W/28/02)
20 ml .... distilled water
20 ml .... glycerol
14 ml .... lactic acid B.P.
16 ml .... phenol, detached crystals B.P.

[2]) Data for prepupal and pupal instars were not plotted because the extraction efficiency for them is low (LEWIS, 1960); they formed curves similar in shape but approximately midway between the larval and adult curves.

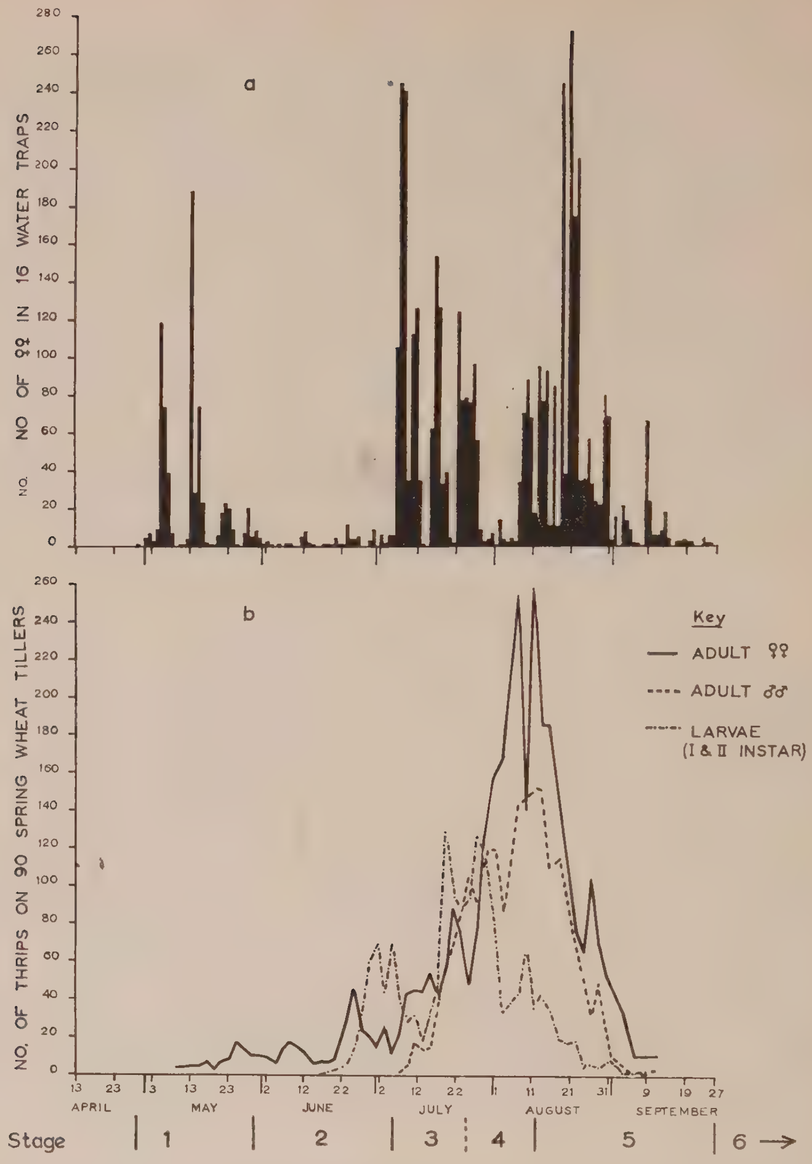

Fig. 3. Numbers of *L. cerealium* caught (a) in water traps and (b) on spring wheat in 1956, showing principal stages in the annual cycle.

ble. At this time numbers on the spring wheat crop were low; in 1956 about 1 ♀ per 8 tillers and in 1957, 1 ♀ per 21 tillers.

In 1957 there were many more on the larger winter wheat and oats tillers than on either spring wheat, rye grass, timothy (Fig. 4 & 5) or cocksfoot. The num-

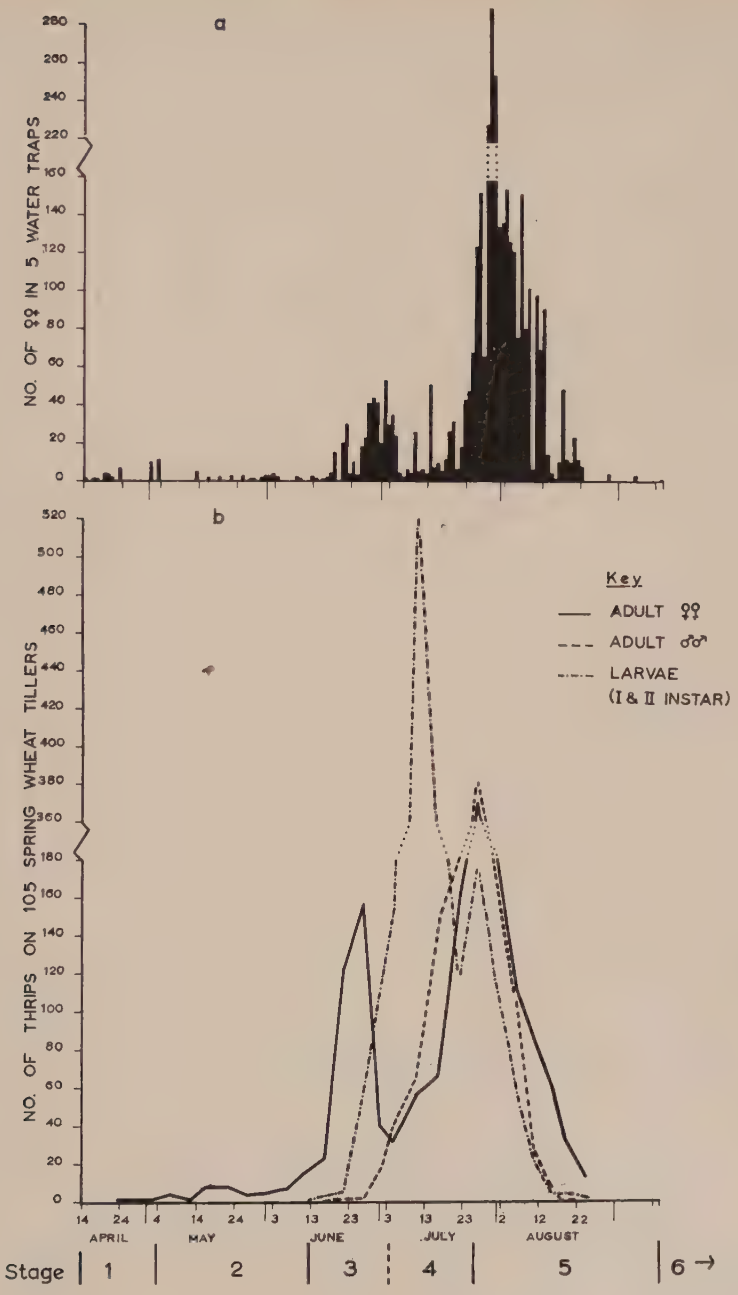

Fig. 4. Numbers of *L. cerealium* caught (a) in water traps and (b) on spring wheat in 1957, showing principal stages in the annual cycle.

bers of females per tiller increased with tiller height: on 29th April 1958 winter wheat tillers were divided into four height categories as follows:— 8—11, 11—14, 14—17 and 17—20 inches, and two samples, each of ten tillers, taken from each category, together yielded 18, 58, 90 and 122 thrips respectively.

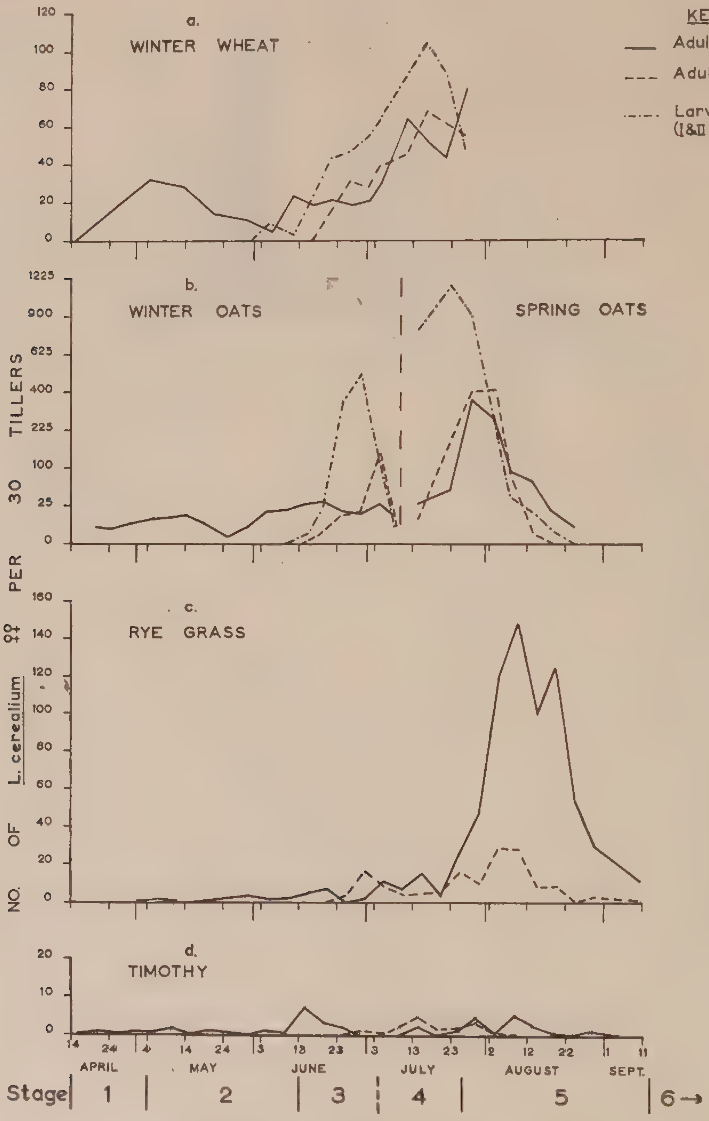

Fig. 5. Numbers of *L. cerealium* on (a) winter wheat, (b) oats, (c) rye grass and (d) timothy, 1957, with principal stages in the annual cycle. (For winter and spring oats the proportions of *L. cerealium* are approximately 30% and 90% respectively).

Thus the different population densities were probably due to differences in plant size[1]), more thrips reaching the taller plants which also offered most shelter.

When feeding began the ovaries soon started to develop; only 2% were immature in females collected from hosts between 19 April and 4 May 1957 but of

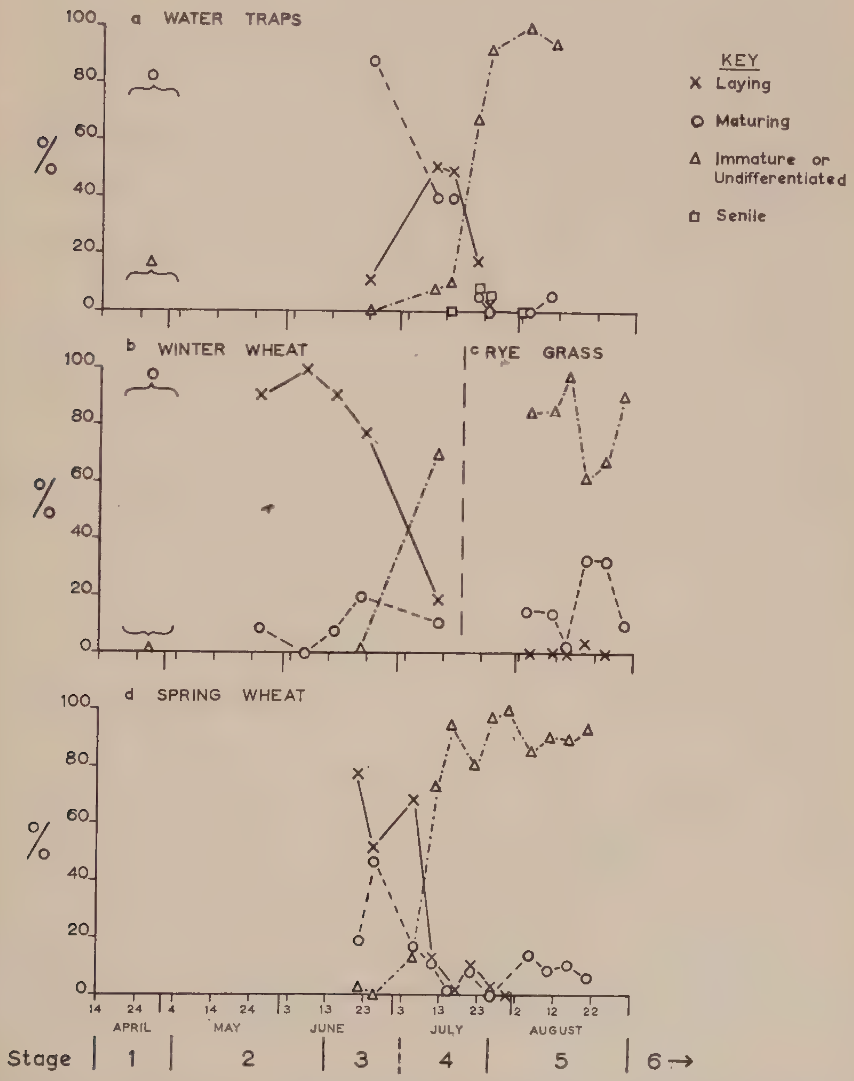

Fig. 6. The percentages of *L. cerealium* females at different stages of ovarian development in the air and on some host plants, 1957.

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[1]) For wheat tillers in this height range tiller height is directly proportional to surface area. (LEWIS, 1958).

those in flight at the same time 17% were still immature (Fig. 6). At the end of the 1958 spring emergence 82% of females from winter wheat were maturing and 18% laying.

Stage 2. Ovarian development and start of extensive oviposition. The spring emergence was followed by about 40 days in which relatively little flight occurred and numbers on the wheat crop remained fairly constant.

In 1957 eggs were first seen on 21st May. Four days later, 91% of the females on winter wheat were laying and there were about 12 eggs per tiller on these plants by 30th May, when there was less than one egg per tiller on the spring wheat crop. Eggs were found only on the leaf blades. Numbers of females on winter oats and wheat were similar. In general, samples of wild grasses yielded more thrips than the corresponding sown grasses, probably because they were larger plants and some had already flowered.

In 1958 a few eggs were noticed on winter wheat on 12th May. Seven days later there were about 12 eggs per tiller on these plants but only 0.2 per tiller on spring wheat.

For study of their oviposition, individual females of *L. cerealium* were kept in cages made from open glass tubes (2 × 3/8 in. diam.) embedded 1/3 in. deep in a plaster of Paris block. The tubes were stoppered with non-absorbent cotton wool and the block moistened daily. This kept the relative humidity inside the tubes high without causing condensation on their walls, and fragments of food stayed fresh in them for 2 days. Twenty females were taken from hibernation in bark on 28th March 1958 and kept on wheat in these cages at 23° C. Thrips began to lay after 12—27 days feeding and the maximum duration of oviposition for an individual was 58 days, during which 108 eggs were laid.

Stage 3. Movement to fresh hosts, appearance of the new generation and death of old females. The second period of flight activity began when the females moved to fresh host plants to continue oviposition. Movement from the earlier infested plants probably started when they became less juicy, making feeding difficult.

On 22nd June 1957, 12% of females caught in flight were laying and the ovaries of 88% were still in a maturing condition. This high percentage of maturing females in flight was contrasted with about 80% of laying females on winter wheat (cf. Fig. 6a & b). Again in 1958 the percentage of laying females in flight (32%) was less than the percentage on wheat (69%). The reason for this difference is not known; perhaps laying females fly less readily.

In 1956 the increase in numbers of adult females on the crop which had reached growth stages 5—7 (Large, 1954) was less marked and more protracted than in 1957, when the infestation was rapidly established; there was only a small increase in numbers on winter wheat and oats. Soon after females moved to the crop many eggs appeared, and in 1957 oviposition was almost completed by the first week in July. Oviposition in stage 2 had been confined to the leaf blades but as these yellowed, first sheaths and then ears were preferred. On 4th July, 1957, 10 spring wheat tillers, each with 3 intact leaves, were dissected, and the eggs and egg shells counted to show the distribution of eggs on the plants since laying began (Table II).

TABLE II

The numbers and distribution of eggs on 10 spring wheat tillers, 4 July 1957.

	No. of eggs		
Position of leaf	Blades	Sheaths	Ears
Upper	59	989	
Middle	24	7	
Lower	0	2	173
Total	83	998	173
% of Total	6.5	79.5	14

Most eggs were laid on the inside of the youngest (upper) leaf sheaths, though prior to stage 3 eggs on spring wheat had been found only on leaf blades.

The continued flight activity after most females had apparently reached the crop (Fig. 4b) suggests movement within it throughout the remainder of the oviposition period. The drop in the number of females on the crop between 26th June and 4th July, 1957 was due to the death of senile females, whose bodies could be found lying on the leaf sheaths and ears. (On 21st and 25th July a few were caught in water traps.). In 1956, no similar drop occurred when the old females died.

Soon after the crop became heavily infested, larval numbers increased. In 1956 a small larval peak appeared late in stage 2 and at the beginning of stage 3, but this was not so in 1957. The earliest new adults produced from eggs laid in stage 2 appeared midway through stage 3. New generation males were first recorded on 5th July, 1956 and 21st June, 1957.

Stage 4. Development of the new generation on cereals. A period with less flight activity during which development of the new generation continued, followed the end of oviposition and the death of most old females. There was no precise date on which this stage started as the larval population increased rapidly whilst some old females were still laying and flying. As they died, there was a rapid increase in the percentage of females which were undifferentiated and immature — the newly emerged generation — and from 15th July, 1957 until harvest about 90% remained immature. Maximum numbers of adults on the crop occurred in 1956 on 10th August (2.9 females and 1.7 males per tiller) and in 1957 on 26th July (3.5 females and 3.5 males per tiller).

On winter wheat slightly fewer of the new generation were produced and by 25th July sampling ceased when these plants became indistinguishable from the spring wheat. Large numbers of larvae appeared on winter oats, until powdery mildew made them dry and brittle, causing their population to collapse by the beginning of July.

Oats sampling was continued on a spring variety where the population curve followed a similar pattern to that on wheat, but there were about 7 times as many larvae and 3½ times as many adults per tiller. A little breeding occurred on rye grass but numbers on the other grasses were very low.

Stage 5. Movement from cereals. Immediately after the peak of adults was reached, in both seasons, numbers of all stages in the crop decreased rapidly. Males died when the ears ripened and females flew off giving a third period of intense flight activity. In 1957 about 90% of these females, both in flight and on the crop, had undifferentiated or immature ovaries and the remainder showed slight development. A similar decrease occurred on spring oats.

At the end of stage 4 and the beginning of stage 5 a comparison of the numbers of *L. cerealium* per ear with ear size was possible because the distribution of thrips on the crop was not greatly influenced by "edge-effects". The mean number of grains per ear at each sampling site was used as a measure of ear "size" (Site D was omitted as ears there were badly damaged by birds). For adult females and males, the numbers taken per site were plotted against the corresponding ear size and a linear regression line was fitted. The regression was significant for females ($P < 0.05$) but not for males.

In 1957, the fall in numbers on the cereals corresponded with a big increase in the number of females on rye grass (Fig. 5c). As little breeding had occurred there, this sudden rise was due to movement from the cereals. The ovaries of some females on the rye grass showed some development; between 19th August and 24th August 32—33% had maturing ovaries and 4% were in laying condition (Fig. 6c), but no eggs were found. There was no increase in numbers on either cocksfoot or timothy.

Stage 6. Hibernation. Flight activity decreased after the females left the crop and grasses; the last flight records were on 26th September 1956 and 11th September 1957. During the winter females were found hibernating in bark, especially on pine and oak trees around the field. Slivers of pine bark, totalling about 8 square feet, collected from the bottom of trunks in February 1957, yielded 96 females in an emergence tin.

On 28th March, 1958 40 females were collected from the bark of an oak tree standing in the oats field. The ovaries of 91% were undifferentiated; the rest were differentiated but the longest rudiment was less than 50 μ. These percentages were similar to those found in females from wheat in August, thus little or no ovarian development appeared to have occurred during the winter. However, by the end of stage 1 in 1958, 18% of females had reached laying condition. It is doubtful whether they would have had time for this had they emerged from hibernation with immature ovaries. This suggests that early-laying females correspond to those whose ovaries had partly developed whilst feeding on grass the previous autumn. The absence of maturing females in the oak bark sampled was perhaps because most thrips there had developed on oats where little ovarian development had occurred late in the season.

COMPARISON OF SEASONS AND HOSTS

March and April in 1957, being milder than in 1956, the spring emergence was consequently about a fortnight earlier; the stages remained about that much earlier throughout the season (Table III).

Many more females were found in Spring 1956 than in Spring 1957, presumably because the earlier winter was more favourable for survival and/or the previous summer population was larger. The difference remained throughout stage 2. The sudden small peak of females on the crop towards the end of stage 2 in 1956 (Fig. 3) coincided with a slight increase in flight activity, so may represent the beginning of movement to fresh hosts. However, the small larval peak immediately succeeding this increase in females, was unlikely to be due to their oviposition because eggs take about 10 days to hatch. These early larvae on the spring wheat were therefore probably from eggs laid by females which had been on the crop since emerging in May; the larger, second larval peak resulting from oviposition after the main influx of females in stage 3. The heavier early spring adult infestation on the spring wheat in 1956 may also account for the absence in that season, of a marked decrease in numbers of females on the crop when the senile ones died; their loss coincided with the emergence of adults from the first larval peak, so no overall decrease was apparent.

TABLE III

The approximate dates of commencement and duration of stages in the annual cycle, 1956 and 1957.

Stage	1956		1957	
	Date of commencement	Duration (days)	Date of commencement	Duration (days)
1	30/4	30	14/4	20
2	30/5	37	4/5	40
3	6/7	19	13/6	21
4	25/7	18	4/7	22
5	12/8	46	26/7	48
6	27/9	199	12/9	221
Total number of days between first and last recorded flight		150		151

Though fewer females emerged in 1957, by the time the new generation had developed (stage 4) numbers per plant were higher than in 1956. This may have been due to more favourable weather conditions in 1957, when there was less rain during stages 3 and 4. In 1956 rain also caused the big day-to-day fluctuations in the aerial population during times of flight activity.

Early in the season most thrips were found on wheat, but later the greatest numbers were on spring oats — perhaps because these had, in general, a slightly higher water content.

Of the grasses sampled, rye grass was the most favoured host but although a little breeding occurred on it, large numbers of thrips were found there only when the wheat became unsuitable, It seems possible that a small second generation would occur on this grass in a favourable season; MORISON (1943) in Scotland found a second generation on oats when they were standing until late September or October. Little breeding occurred on timothy; the small peaks in numbers of females on it coincided with the second and third periods of flight activity and the thrips were probably blown there as they moved on and off the crop. On cocksfoot plots only 23 females and 4 males were taken throughout the season. Numbers on them might have been higher if the plants had flowered (on 21st May, 1957, 9 females were found on 30 wild cocksfoot inflorescences) but evidently the leaves of this grass are an unsuitable host.

THE WATER CONTENT OF EARS AND THEIR THRIPS POPULATION

During stage 5, in 1957, the crop was sampled to investigate the relationship between water content of ears and numbers of thrips on them. All samples were taken on the same day because thrips soon leave the crop when it starts to ripen. "Wet" green ears and "dry" brown ears were selected on a colour basis, so that the 15 ears in each of the 18 samples were uniform. Samples were collected over a large area to avoid any possible influence of site. After thrips were extracted the mean water content of each sample was determined: samples were halved,

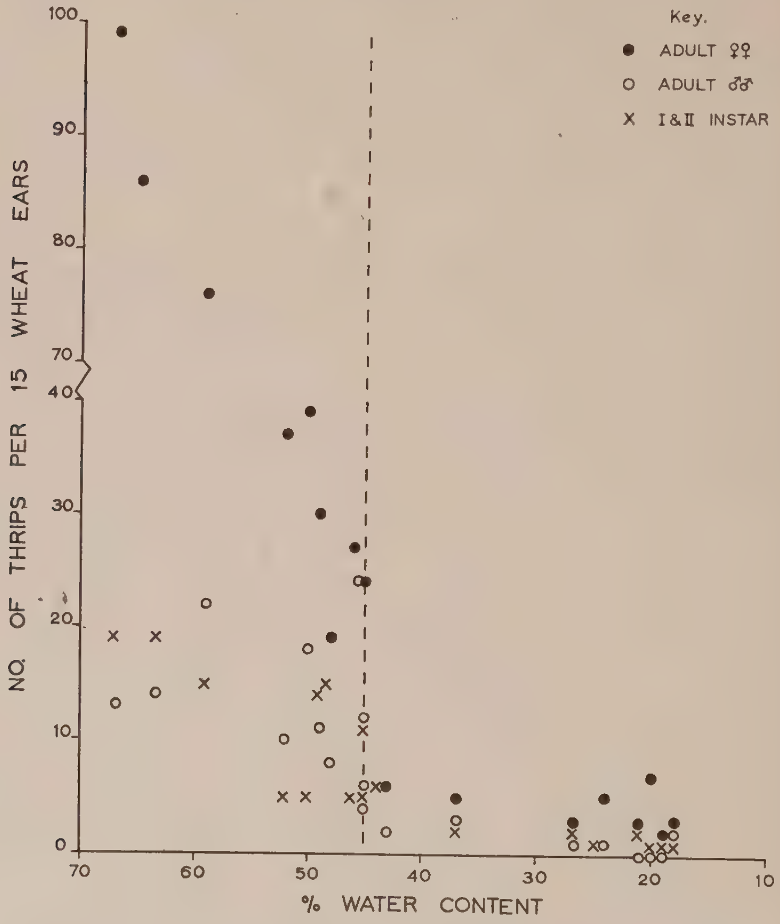

Fig. 7. The relationship between numbers of *L. cerealium* and the water content of ears.

each half weighed and dried to constant weight at 103° C. The loss in weight expressed as a percentage of the original weight was taken as percentage water content. The standard error of these determinations was ± 1.7%.

The number of females per ear and ear size are related at this time of year (see P. 200), so numbers of females were corrected for the mean sample size.

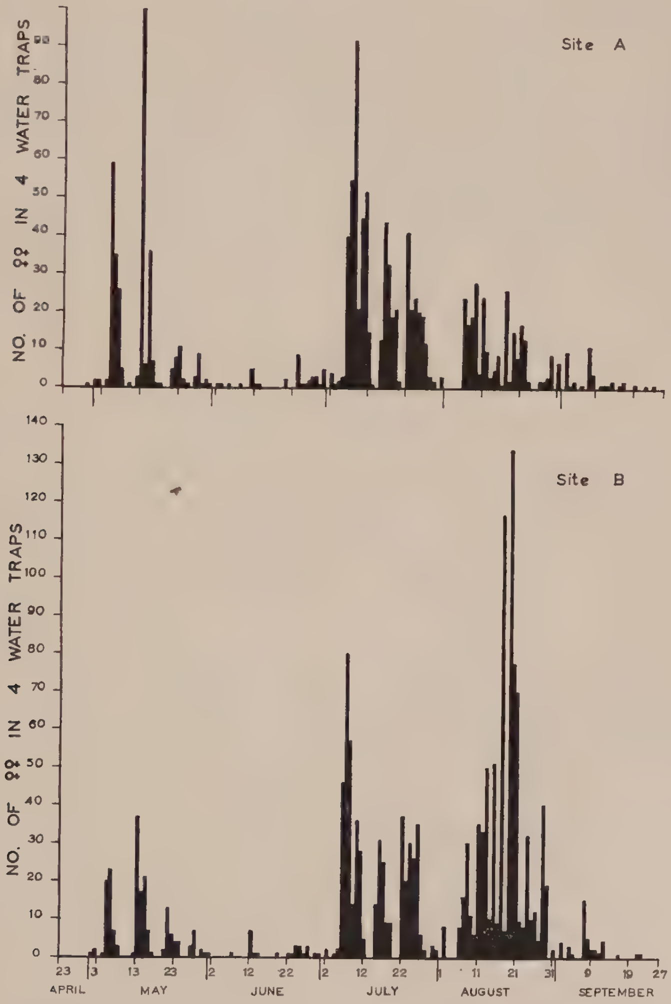

Fig. 8. Daily catches of *L. cerealium* females in water traps at sites A and B in 1956.

Numbers of thrips were plotted against corresponding water contents (Fig. 7). Ears with less than about 45% water content were not favoured; probably the thrips are unable to feed.

"EDGE" EFFECTS

When females first reached the spring wheat crop in 1956, most were found along the S.W. edge; 46% of all females collected from sites A. E, J, F and C in May were from A.

Similarly, more thrips were collected in the water traps at A than at B (Fig. 8). Distribution over the field gradually became more even as the season progressed. When females left the crop, the traps at B caught most.

The winds throughout the season were predominantly westerly; thus thrips appear to have been deposited mainly on the S.W. edge of the crop or on the adjoining rough-grass strip, which may have had a combing-out effect; from there, the infestation gradually spread downwind into the crop. Late in the season the wheat rather than the surrounding grasses became the main source of thrips so the wind blew most thrips over the more leeward traps at B. The catch-direction roses (Fig. 9) indicate the general trend of movement over the field in the 1956 and 1957 seasons. In the latter, the winds during periods of flight activity were variable, so no marked edge-effects appeared.

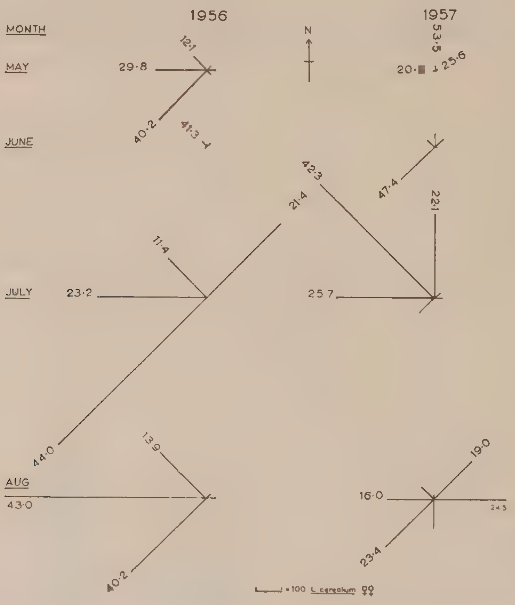

Fig. 9. The monthly totals of *L. cerealium* females caught in water traps in different wind directions. The principal catches are expressed as a percentage of the total monthly catch.

Acknowledgements. It is a pleasure to thank Prof. O. W. Richards F.R.S., who was my supervisor throughout the course of this work, and Dr. G. D. Morison of the North of Scotland College of Agriculture for his advice and criticism. Dr. R. E. Blackith and Mr. H. F. van Emden of Silwood Park kindly translated foreign papers. I am indebted to Mr. H. Goto of the Imperial

College of Science and Technology for permission to publish his polyvinyl lactophenol formula.

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SOMMAIRE

CYCLE ANNUEL ET DISTRIBUTION DANS LES CHAMPS DE BLÉ DE LIMOTHRIPS CEREALIUM *HALIDAY (THYSANOPTERA)*

Les populations terrestres et aériennes de l'espèce *L. cerealium* (Thysanoptères) étaient étudiées simultanément: des dissections ovariques étaient faites pour discerner les générations différentes. On peut arbitrairement diviser la vie de ces insectes en six périodes, basées principalement sur l'activité aérienne: au printemps les femelles se nourrissent pour la plupart d'herbes et de céréales d'hiver; en juin et juillet beaucoup d'elles passent sur les céréales de printemps, où elles pondent la plupart des oeufs dans les gaines des jeunes feuilles. Les femelles de la première génération laissent les céréales en aôut pour se nourrir d'herbes avant d'hiverner.

Les Thysanoptères abandonnaient les épis dont la teneur en eau était approximativement moins de quarante-cinq pour cent. Quand le vent soufflait constamment du même côté l'infestation initiale était plus forte au bord du champ qui se trouvait exposé au vent.

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A COMPARISON OF WATER TRAPS, CYLINDRICAL STICKY TRAPS AND SUCTION TRAPS FOR SAMPLING THYSANOPTERAN POPULATIONS AT DIFFERENT LEVELS

BY

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Thysanoptera drifting over and around a wheat field were trapped using black, green and white water traps, black cylindrical sticky traps and suction traps. Certain *Taeniothrips* spp., *Thrips* spp., and *Aeolothrips* spp. were strongly attracted to white but not to green or black; *Limothrips cerealium* Hal., *Chirothrips manicatus* Hal., and *Stenothrips gramium* Uzel were not markedly attracted to any of these colours. At crop level suction traps gave more consistent catches[2] than either sticky or water traps; at higher levels, catches from sticky traps were more consistent than those from water traps. Up to 48 ft the aerial density of all species decreased with height but the rapidity of the decrease differed between species.

Various impaction traps which depend for their catch on the natural, variable wind, have been used to catch thrips. KÖRTING (1931) caught *Limothrips cerealium* Hal. and *Haplothrips aculeatus* (Fabricius) on sticky posts up to 5.42 metres tall. JOHANSSON (1946) trapped many species up to 16 metres over cereal fields using a trap consisting of a gauze screen against which the thrips were blown and then fell into a collecting jar. A vane kept the screen facing the wind.

Catches by these types of trap would depend mainly on the density of thrips in the air and on the wind flowing past the traps, conditions which vary between species, times and heights. Thus data collected under different conditions may not be directly comparable unless catches are consistent and represent similar proportions of the population sampled. No indication of such details in respect of their traps are given by either KÖRTING, or JOHANSSON.

This paper describes traps used to catch thrips drifting over and around a wheat field, particularly those species feeding on Gramineae. A large proportion of *L. cerealium* and perhaps *Limothrips denticornis* Hal. probably originated within the crop, but the majority of other species were caught as they drifted over the crop from other sources. Catches from certain colour traps were compared and an attempt has been made to assess the reliability of the trapping methods and to indicate their value for different aspects of population studies on thrips.

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[2] See footnote on p. 208.

TRAPPING METHODS

Water traps

The traps were tins, 11½ × 7½ × 2½ in., painted either white, black or green inside and black outside. White was selected to reflect all colours, green[1]) to correspond with host plants and black because it was thought least likely to be attractive.

A few drops of 40% formaldehyde and about 50 ml of "Stergene" were added to each bucket of water used in the traps. The detergent caused most thrips to sink and so minimised the possibility of their escaping by drifting to the sides and climbing out: the formaldehyde prevented algal and fungal growth. The water level was kept about ½ in. below the rim and thrips were collected with a pipette. Each trap was fitted into a metal rack bolted or clamped to an adjustable stand.

Sticky traps

These traps consisted of a black metal pipe 9 in. long × 1 in. diam., on an adjustable stand. A glass boiling tube, 10 × 1¼ in. diam., covered clear tree-banding grease, "Orbite", was inverted over the metal pipe. Exposed tubes were collected and replaced with freshly greased ones. In the laboratory tubes were slid over a white pipe and thrips removed from the bottom 6 in. Any grease on the thrips was dissolved in turpentine before they were identified, and the tubes washed in paraffin.

Suction traps

Suction traps (Taylor, 1951), which sample a constant volume of air and are therefore probably non selective were used as a standard with which to compare the water traps. Their disc-dropping mechanism was not required for this as water traps were only emptied daily, but it was successfully used for separating hourly catches of thrips on other occasions (Lewis, 1958). The specified copper gauze cone, through which thrips could crawl was replaced by a nylon cone (mesh 65 × 71 threads per inch) which slightly reduces the airflow (Taylor, 1955). In order to kill certain *Taeniothrips* spp. and *Thrips* spp., it was necessary to increase the concentration of pyrethrum solution applied to the discs to about four times that recommended for aphids (Johnson, 1950). When the wheat was small the orifices of the traps were placed at crop level by lowering the cones into a long slit-trench.

COMPARISON OF TRAPPING METHODS

i) *Comparison of black, green and white water traps and of suction traps*

Thrips were trapped in a wheat field with black, green and white, water traps and suction traps. Two traps of each type were arranged equidistantly in a circle of 80 feet diam., where the crop was evenly grown. They were kept adjusted to crop level throughout the trapping periods.

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[1]) The shade of green was produced by mixing green and yellow paint — Naylors' "Belco" — to give a colour matching a young wheat plant. This corresponded to Scheeles green (Wilsons Colour Chart II ref. 800/1).

The need for an electric power source meant that some traps had to be within a few yards of the crop edge, but as far as possible one trap of each type was placed nearer the edge than the other, so that when catches from like traps were added, positional effects would be reduced.

Thrips were trapped in each of the three periods of *Limothrips cerealium* flight activity (Lewis, 1959) (Table I). The suction traps caught great numbers, so to reduce laborious identification and counting, two were used in the second

TABLE I

The number of thrips caught in black, green and white, water traps and in suction traps and the percentage of the total catch represented by each species.

21/4—4/5*

Species	Black	Green	White
L. cerealium ♀♀	18	15	16
C. manicatus ♀♀	9	15	11
Taeniothrips spp. ♀♀	—	3	2
Thrips spp. ♀♀	17	14	27
Aeolothrips spp. ♀♀ & ♂♂	—	—	3
Melanothrips spp. ♀♀	—	—	1

* Percentages were not calculated as total catches were small.

25/6—2/7

Species	Black		Green		White		Suction*	
	No.	%	No.	%	No.	%	No.	%
L. cerealium ♀♀	173	60.7	252	66.7	155	2.5	4,815	62.9
L. denticornis ♀♀	2	0.7	16	4.2	3	0.1	173	2.3
C. manicatus ♀♀	28	9.8	26	6.9	31	0.5	710	9.3
S. graminum ♀♀ & ♂♂	9	3.2	10	2.5	9	0.15	191	2.5
Anaphothrips obscurus ♀♀	1	0.4	—	—	2	0.05	36	0.5
Frankliniella spp. ♀♀ & ♂♂	7	2.4	11	2.9	4	0.1	275	3.6
Taeniothrips spp. ♀♀ & ♂♂	53	18.6	46	12.2	5,474	89.9	1,045	13.6
Thrips spp. ♀♀ & ♂♂	11	3.8	12	3.2	370	6.1	371	4.8
Aeolothrips spp. ♀♀ & ♂♂	1	0.4	5	1.4	37	0.6	28	0.4
Unidentified	—	—	—	—	—	—	11	0.1
Total	285		378		6,085		7,655	

* 2 traps.

3/8—11/8

Species	Black		Green		White		Suction*	
	No.	%	No.	%	No.	%	No.	%
L. cerealium ♀♀	374	96.9	360	96.3	339	90.1	4,641	91.5
L. denticornis ♀♀	2	0.5	2	0.5	4	1.1	35	0.7
C. manicatus ♀♀	8	2.1	9	2.4	16	4.3	190	3.7
A. obscurus ♀♀	—	—	—	—	5	1.3	78	1.5
Frankliniella spp. ♀♀	1	0.25	1	0.3	3	0.8	87	1.7
Taeniothrips spp. ♀♀	—	—	—	—	1	0.3	16	0.3
Thrips spp. ♀♀	—	—	—	—	—	—	19	0.4
Aeolothrips spp. ♀♀	1	0.25	2	0.5	8	2.1	7	0.2
Total	386		374		376		5,073	

* 1 trap.

period, and only one in the last. For analysis of numbers caught in different traps a square root transformation was used and the variation between colours was compared with the variation between traps within colours. The proportions of species caught in the traps were compared by taking the angular transformation and again comparing the variation between types of trap with the variation between duplicate traps ($P > 0.05$).

Results

21st April—4th May. The numbers of *L. cerealium* and *Chirothrips manicatus* caught in black, green and white traps were similar; most *Thrips* spp. were caught in white traps but the difference was not significant.

25th June—2nd July. In the water traps significantly more *L. cerealium* were caught in green traps but the numbers of *C. manicatus* and *S. graminum* in any colour were not significantly different. Thus there is a suggestion that *L. cerealium* was differentially attracted to green at this time. However the percentages of this species, *C. manicatus* and *S. graminum* taken in the black, green and suction traps were not significantly different, so perhaps the greater number of *L. cerealium* in the green traps was due to a bigger total catch rather than to differential attraction to green. The increased catch may have been due to the position of the traps in relation to the crop edge. More *L. denticornis* were caught in green traps than in black or white but the total catch was small and half the number in green traps were caught on one day. The numbers of *Taeniothrips* spp. and *Thrips* spp. in black and green traps did not differ significantly but many were caught in the white traps in which these two genera together accounted for 96% of the total catch. White traps caught significantly more *Aeolothrips* spp. than green or black, but other species were insufficiently numerous to give a reasonable comparison between traps.

3rd August— 11th August. Most *L. cerealium* were caught during this period but there was no significant difference between numbers in any colour. White traps caught slightly more *Aeolothrips* spp., *Anaphothrips obscurus* Müller, and *C. manicatus* than black or green, so the cumulative effect of these small catches was sufficient to lower significantly the percentage of *L. cerealium* caught in them. Comparison of the percentages of each species caught in the different water traps and in the suction trap by the method described above was not possible as only one suction trap had been used. The proportion of *L. cerealium* in the black and green traps appears slightly higher than in the suction trap. This may be because the latter had a much larger total catch and thus gave a truer estimate of the less common species in the air than the water traps.

Data from the three trapping periods showed that certain *Taeniothrips* spp., *Thrips* spp., and *Aeolothrips* spp.[1]) were strongly attracted to the white water traps but not to black or green, and that *L. cerealium, C. manicatus,* and *S. graminum* were not markedly attracted to either black, green or white. The species attracted to white, with the exception of the predaceous *Aeolothrips* spp. feed mainly on a wide range of dicotyledonous flowering plants, whereas the unattracted species are all grass-feeders. In this respect it is noted that HEATHCOTE (1957) and EASTOP (1955) found that grass and sedge-feeding aphids were attracted less to yellow traps than aphids feeding on dicotyledons.

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[1]) Mainly *Taeniothrips vulgatissimus* (Haliday), *Taeniothrips atratus* (Haliday), *Thrips major* Uzel, *Thrips fuscipennis* Haliday (Det. G. D. Morison), and *Aeolothrips tenuicornis* Bagnall.

ii) *The consistency of catches*[1])

A measure of the consistency of catches obtained by the different types of trap is given by their coefficient of variation. This was calculated for each type of trap at crop level and for sticky and water traps at 6 feet. As there was no differential attraction to black traps between the species studied, the coefficient of variation for catches by the black suction and sticky traps was based on total thrips caught. For the white water traps a separate coefficient of variation was calculated for each species. (Table II).

TABLE II

The coefficient of variation for catches in black suction traps at crop level, and in black, sticky and white water traps, at crop level and 6 feet.

Type of trap	Species	Coeff. of var. for 2 heights Crop level	 6 ft.	No. of replicates
Black suction	All Thysanoptera	± 7.8%	—	8
Black sticky cylinders	All Thysanoptera	± 21.4%	± 20.2%	25
White water	*L. cerealium*	± 27.8%	± 42.8%	25
	C. manicatus	± 28.6%	± 56.4%	25
	Taeniothrips spp.	± 26.8%	± 27.8%	25
	Thrips spp.	± 29.4%	± 37.6%	25

The suction trap catches were most consistent; sticky trap catches were less so and water trap catches least consistent. Sticky trap catches were about equally consistent at crop level and at 6 feet. The consistency of water trap catches at crop level was similar for all species but it decreased for some, particularly those unattracted to white, at 6 feet.

The sticky cylinders would interfere much less with the airflow past them than would the angular water traps, and the difference in the reliability of the two methods is perhaps due to the amount of turbulence the different traps caused.

iii) *The relative efficiency of the traps*

The non-attractive water traps caught between approx. 1/12 and 1/30 the number of thrips caught in the suction trap, but attractive water traps (for *Taeniothrips* spp.) caught about 5 times as many thrips as the suction trap.

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[1]) The coefficient of variation $= \frac{\text{Standard Error}}{\text{Mean}} \times 100$. By the word "consistency" is meant the measure of agreement between catches from like traps exposed to a given population density of thrips; the more "consistent" the catch, the smaller is the coefficient of variation. The coefficient of variation was used as an expression to compare consistency of catches, because it is independent of total numbers caught.

The true efficiency of the traps, i.e. the proportion of total thrips in the air sampled retained on them, is not known. For sticky traps, particularly, this may increase with increasing wind speed (GREGORY, 1951), but to calculate this the insects would have to be considered as inert particles, which is unrealistic.

THE VERTICAL DISTRIBUTION OF THRIPS IN THE AIR

Preliminary observations made on the vertical distribution of 4 common species using white water traps at crop level and 6 feet (Table III) showed that numbers of *L. cerealium*, *C. manicatus* and *S. graminum* were greatest in the lowest traps but *L. cerealium* was distributed more evenly between the two heights than the other two species. Most *L. denticornis* were caught at 6 feet.

More detailed observations on vertical distribution were made by trapping with white water and black sticky traps on a latticed tower, 50 feet high, sited on a small spur about 200 yards from the nearest cereals. The vegetation surrounding the tower was a mixture of poor grassland (mainly *Agrostis* spp. and *Festuca* spp.) and herbaceous weeds. The nearest trees were about 70 yards away. Sticky and water traps were placed at 3, 6, 12, 24 and 48 feet, above ground level; those at the lower two levels were fixed on stands 10 yds. away from the base of the tower. This was to avoid any interference in the wind flow which a nearby metal box housing meteorological instruments may have caused. As the sticky traps were held vertically they were fixed so that they extended for half their length above and half below the stipulated height, e.g. the functional surface of the trap at "3 feet" extended from 2 ft 9 in. to 3 ft 3 in. The water traps needed refilling about every four days and thrips were collected at approximately 10-day intervals from 27th April—23rd August, 1957.

TABLE III

Numbers of 4 species caught at crop level and at 6 feet during their respective periods of flight activity in 1956.

Species	Crop level	6 feet	Total
L. cerealium	1269	1064	2333
L. denticornis	64	79	143
C. manicatus	1619	715	2334
S. graminum	243	98	341

In order to give a true picture of vertical distribution the raw data needs conversion from catch to insect density (TAYLOR, 1959). Values for the relative wind speed at each trapping height are needed for this. They were obtained from a wind profile suggested by JENSEN (1954), using the height (d) of the vegetation at the tower site for his parameter (d). Though this profile may not be exact due to the slightly uneven height of the vegetation and its continued growth throughout the season, it must closely approximate to the mean relationship between wind speed and height over a period as long as the trapping period. From the relative wind speed values, correction factors for each trap height were obtained. Catches × correction factor gave an estimate of relative density at each height (Tables IV & V).

TABLE IV

Numbers of thrips caught on sticky traps and the relative density (D_r) between 3 feet and 48 feet

Height above ground level (ft)	Height above bottom trap (ft)	Corr. Factor	*L. cerealium*		*L. denticornis*		*C. manicatus*		*Taeniothrips* spp.		*Thrips* spp.	
			Nos.	D_r	Nos.	D_r	Nos.	D_r	Nos.	D_r	Nos.	D_r
3	0	×8	113	904	13	104	104	864	64	512	82	656
6	3	×2	144	288	31	62	38	76	30	60	94	188
12	9	×1.5	120	180	23	34.5	25	37.5	39	58.5	58	87
24	21	×1.1	119	130.9	26	28.6	17	18.7	17	18.7	40	44
48	45	×1	67	67	19	19	5	5	8	8	29	29

TABLE V

Numbers of thrips caught in water traps and the relative density (D_r) between 3 ft and 48 ft.

Height above ground level (ft)	Height above bottom trap (ft)	Corr. Factor	*L. cerealium*		*L. denticornis*		*C. manicatus*		*Taeniothrips* spp.		*Thrips* spp.	
			Nos.	D_r	Nos.	D_r	Nos.	D_r	Nos.	D_r	Nos.	D_r
3	0	×8	88	704	9	72	90	720	1030	8240	398	3184
6	3	×2	85	170	22	44	20	40	241	482	106	212
12	9	×1.5	107	160.5	22	33	24	36	206	309	101	151.5
24	21	×1.1	135	148.5	23	25.3	22	24.2	97	106.7	44	48.4
48	45	×1	77	77	31	31	21	21	50	50	20	20

In order to prevent leaves and seeds fouling the lowest traps these had to be a few inches above the top of the vegetation. This was about 75 cm (2 ft 3½ in.) tall, so the bottom of the lowest sticky trap was 2 ft 9 in. above ground level. For analysis of the data the lowest trap was regarded as being at 0 feet (*i.e.* as near to the vegetation as practicable to avoid contact) and the heights of the other traps taken as height above it. The effective trapping heights thus became 0, 3, 9, 21, and 45 feet.

As sticky trap catches maintain their consistency with increasing height, at least up to 6 feet (see p. 205) they were considered likely to give the most reliable catches at higher levels. For species listed in Table IV, log. (relative density) was plotted against log. (height + 1), (one was added as the lowest trap was regarded as being at 0 feet) and a linear regression line fitted whose coefficient, (b) gives a simple measure of the density profile (JOHNSON, 1957). For each of the five sets of data a significant regression of log. (relative density) on log. (height + 1) was obtained. A pooled regression was then fitted to all these data and an analysis of variance performed upon them, from which it was concluded that there were significant differences ($P < 0.05$) between the regression coefficients (Table VI). This means that the density of all species decreases with height but not in all cases with the same rapidity.

TABLE VI

Regression coefficients (b) of log. (relative density) on log. (height + 1)

	b
C. manicatus	—1.2644
Taeniothrips spp.	—1.0300
Thrips spp.	—0.8276
L. cerealium	—0.6458
L. denticornis	—0.4449

The least significant difference between regression coefficients is 0.2269 ($P < 0.05$).

Data from the water traps for *L. cerealium. L. denticornis* and *C. manicatus* were treated in the same way and the regression coefficients obtained from the two trapping methods for each species compared as described above. For *L. cerealium* and *C. manicatus* the profiles obtained by the two methods were similar, though the standard error for the water trap data was greater, but the profiles appeared significantly different for *L. denticornis* (Table VII). As the two types of trap were sampling the same density profile, one must have been less reliable than the other. For reasons given this was probably the water trap.

TABLE VII

Regression coefficients (b), and standard error (Sb) for relative density profiles based on sticky and water trap catches

		b	S_b
L. cerealium	Sticky	—0.6458	± 0.0469
	Water	—0.5049	± 0.1153
C. manicatus	Sticky	—1.2644	± 0.1152
	Water	—0.8667	± 0.2586
L. denticornis	Water	—0.4449	± 0.0255
	Sticky	—0.2498	± 0.0607

DISCUSSION

It is not possible to assess the true visual response of thrips to colours unless the distribution curves of spectral energy for the pigments used are known. Nevertheless, results from the black, green and white water traps show that certain species are differentially attracted to the white. The response to colours other than these is unknown but many species may exhibit differential attraction to some colour. Clearly, if a trap is differentially attractive to any species in a mixed population, it cannot be used to give a true estimate of the relative proportions of the various species in that population, unless it has been standardised against a non-selective trap (cf. Fig. 1).

Suction traps, sampling a constant volume of air, gave the most consistent catches, and may be assumed to be non-selective. Thus they are best used to give an estimate of the true density of thrips in the air and of the correct proportions of different species in the aerial population at a given height. Specimens were clean and easily identifiable on collection, though often slightly desiccated in dry weather and unfit for internal dissection. Their catches from dense populations

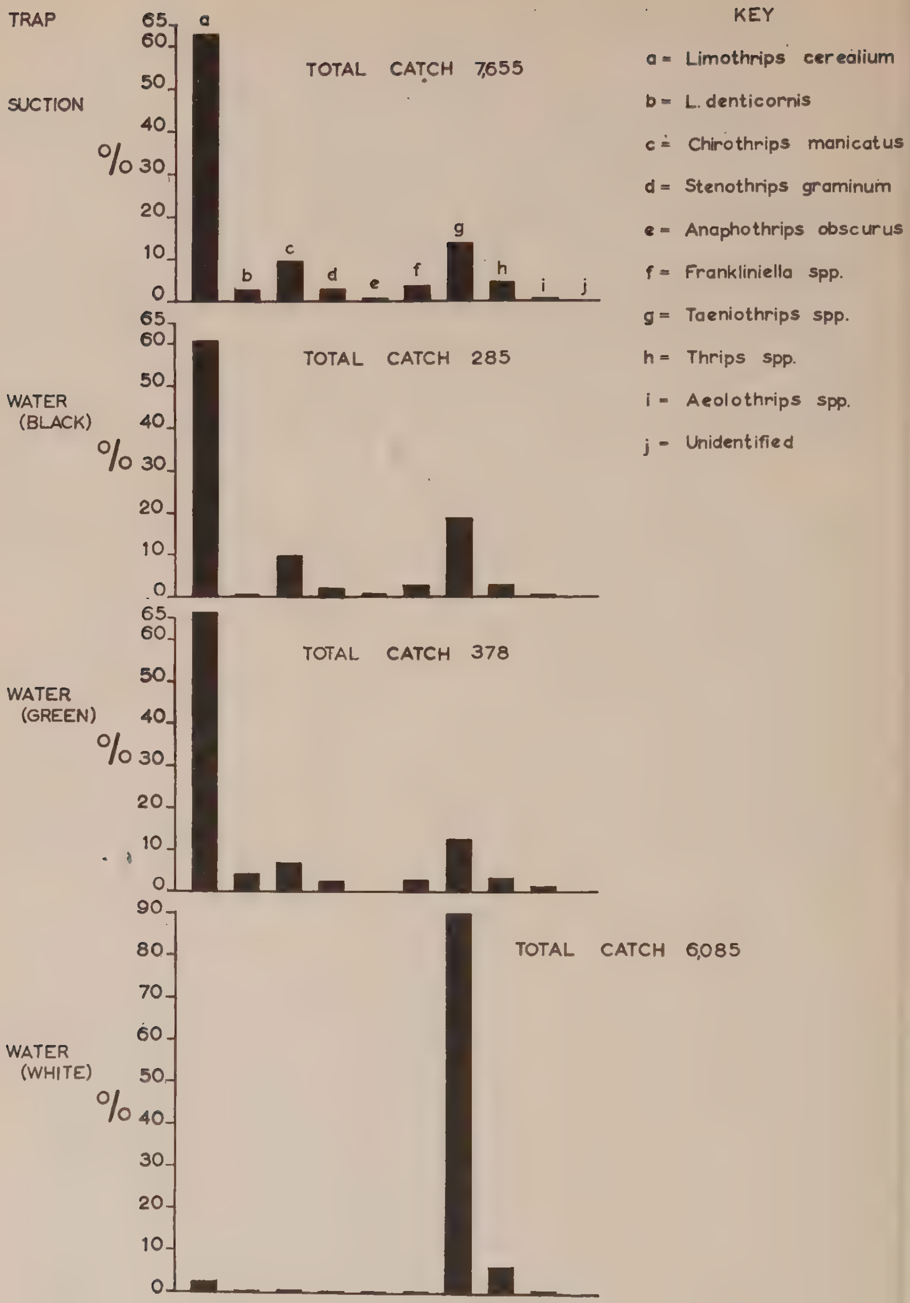

Fig. 1. Percentages of different species caught in a suction trap, and in black, green and white water traps 25th June—2nd July 1957.

are very large resulting in much laborious identification and counting, which limits the number of traps to which proper attention can be given. The traps are expensive and need an electric power source, so their use in many field situations is prevented.

With the disc-dropping mechanism, the traps are invaluable for studying the flight activity of thrips throughout 24 hr periods.

Thrips caught in water traps are easily collected with a pipette in a readily indentifiable condition fit for dissection if collected within 24 hours, but the catches are inconsistent, especially for non attracted species at higher levels. For this reason they are best used at vegetation level where the wind speed is low and where their angular outline will cause least turbulence in the air flowing past them. They are unreliable for quantitative observations on vertical distribution. As unattractive water traps caught far fewer individuals (between $^1/_{12}$ and $^1/_{30}$) than suction traps when exposed to the same population density, large numbers of these traps can be managed and so distributed throughout a wide area to detect the relative amount of flight activity in different places (LEWIS, 1959).

Attractive water traps, which in some cases caught up to approx. 5 times as many specimens as suction traps, would be useful for estimating the relative abundance of less common species, provided the thrips are attracted to the same extent at all times during their annual cycle, as well as for behaviour studies and the collection of large numbers for dissection purposes.

Catches on the sticky cylinders were laborious to sort and identify, and specimens were unfit for dissection, but compared with water traps for quantitative work especially at higher levels, these disadvantages were outweighed by their greater precision. Attractive sticky traps were not used.

The linear relationship fitted to log. (relative density) and log. (height + 1) is not meant to impart a rigid form to the density profiles but it satisfactorily demonstrates two aspects of the vertical distribution which are of importance when considering catches from different heights.

i) For all species density decreases with height. The wind near the ground is mostly highly turbulent (SUTTON, 1953) and both horizontal and vertical components will affect an insect in flight. During the trapping period continuous records of the mean hourly wind speed at approximately vegetation level were kept (to be published elsewhere). Between 9 and 19 hours G.M.T. when most flight occurred the wind speed exceeded 1.5 m.p.h. for 94% of the time and 3 m.p.h. for 31%. At these wind speeds such weak flying insects as thrips, whose approximate flight speed measured over distances of 1—2 metres (3.3—6.6 ft) is 0.5—0.75 m.p.h. (LEWIS, 1958), would be subject to much vertical as well as horizontal displacement. Thus the general diminishing form of the density profile must be partly due to air movement, in which case thrips cannot "select" a height at which to fly. Maximum numbers may be caught some distance above vegetation level where the "flow" of insects past the trap (i.e. insect density × wind speed) reaches a maximum, but this does not mean that most thrips are flying at that height. KÖRTING's (1931) conclusion that thrips " apparently prefer a flight above the height of about 1 metre", supported by JOHANSSON (1946), was based on data which had not been corrected with regard to the relative speed of the wind at different heights. When KÖRTING's catches are corrected to relative density, it has been shown that density diminishes with height as expected (TAYLOR, 1959). My results agree entirely with this.

ii) Though the density decreased with height, the rapidity of the decrease varies between species. *L. denticornis* and *L. cerealium* were distributed more evenly between 0 and 45 feet, than *C. manicatus* which was much more concentrated near the ground (The profiles for the genera *Taeniothrips* and *Thrips* include many species and are difficult to interpret).

From a sampling point of view these differences indicate that traps at the same height may be exposed to different proportions of the population of each species, and that traps at different heights may catch species from a mixed population in different proportions.

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SOMMAIRE

COMPARAISON DE PIÈGES À EAU, DE PIÈGES CYLINDRIQUES À GLU ET DE PIÈGES À ASPIRATION POUR L'ÉCHANTILLONNAGE DES POPULATIONS DE THYSANOPTÈRES À DIFFÉRENTS NIVEAUX

Les Thysanoptères qui flottaient autour et au-dessus d'un champ de blé étaient attrapés par le moyen de pièges à l'eau noirs, verts ou blancs, de pièges cylindriques qui étaient visqueux et noirs et d'aspirateurs. De certaines espèces des genres *Taeniothrips, Thrips* et *Aeolothrips* étaient très attirées par le blanc mais pas par le vert ni le noir: les espèces *Limothrips cerealium* Hal., *Chirothrips manicatus* Hal., et *Stenothrips gramium* Uzel n'étaient pas notablement attirées par chacune de ces couleurs. Au niveau des épis les données obtenues avec les aspirateurs étaient plus justes que ne l'étaient celles obtenues avec les pièges à glu ou les pièges à eau: plus haut les pièges à glu indiquaient des données plus justes que les pièges à eau. Jusqu'à quinze mètres environ de hauteur la densité aérienne de toutes les espèces diminuaient progressivement, mais la rapidité de la diminution était différente entre les espèces.

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RESISTANCE PATTERNS TO VARIOUS ORGANOPHOSPHORUS INSECTICIDES IN SOME STRAINS OF HOUSEFLIES

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The susceptibility of one susceptible and six resistant strains of houseflies to 13 organophosphorus compounds was measured with a contact method. Five of the strains had been selected, both in the field and in the laboratory, with diazinon, parathion or malathion. One strain had been strongly selected with γ-BHC. The susceptibility of the strains is shown in Fig. 1. The BHC selected strain had only a low level of resistance to organophosphates. Higher levels were present in all organophosphate selected strains, each of which exhibited its own characteristic resistance pattern. This indicates that a relatively large number of resistance mechanisms may be present.

As resistance to the thiophosphates is of the same order of magnitude as that to their oxygen analogues, it follows that the resistance mechanism is not a slower conversion of the thiophosphates into the phosphates. Resistance to a number of dimethyl compounds is low, and little difference is present between the resistant strains. This resistance is probably brought about mainly by less specific factors. The genetical basis of the low aliesterase content of the phosphate resistant strains is discussed.

Development of resistance in the housefly to certain organophosphorus compounds is already a well known phenomenon. Strains have been found with high levels of resistance to parathion (Keiding, 1956) diazinon (Keiding, 1956; Sacca, 1957; Labrecque, Wilson & Gahan, 1958) malathion (Labrecque & Wilson, 1957; Kilpatrick & Schoof, 1958) and dipterex (Labrecque et al., 1958). The present paper deals with the resistance patterns to various organophosphorus compounds of some of these strains.

Such patterns may in the first place reveal which kind of compound might replace the insecticides that are no longer effective.

Secondly some information may be obtained concerning the problem whether or not the resistance mechanisms of strains selected with different substances and in different parts of the world depend on similar genetical and physiological factors. It is, of course, important to know whether the insects have many defence mechanisms to rely upon, or whether they are more restricted in their possibilities.

In the third place some information can be obtained on the nature of the physiological defence mechanisms present, provided that a suitable set of insecticides has been chosen for the experiments.

Part of this study has already been published in Dutch (Oppenoorth, 1958b). A study quite similar to ours was carried out by Busvine (1959) who used another test method. Two of his strains have also been used in our study (his Danish strain = D, his Italian strain = C).

MATERIAL AND METHODS

Strains of houseflies

The following strains have been used:

S A susceptible strain that has been kept for many years in the laboratory.

U_2 Originating from Uruguay, kept for many years at the laboratory under strong γ-BHC pressure.

D Collected in Denmark (KEIDING, 1956) after the use of parathion (strain 79b) and kept under parathion pressure since.

A Collected in Denmark by Dr. KEIDING after the use of diazinon (strain 150b) and kept under diazinon pressure since.

F Same characteristics as A (strain 203a).

C Collected in Italy (SACCA, 1957) after the use of diazinon, and kept under diazinon pressure since.

G Collected in Georgia, U.S.A. (FAY, KILPATRICK & MORRIS, 1958) after the use of malathion, and kept under malathion pressure since (= Bethesda 45 colony).

Breeding and selection

Flies were reared in a medium of 100 g whole milk powder, 100 g yeast powder and 20 g agar per l of water and used ½—3 days after emergence. The resistant colonies were kept under light selection pressure in order to prevent loss of resistance. Except for strain U_2 no attempt was made to increase the resistance by continuous heavy selection, because this could perhaps induce an unspecific, so called "vigour tolerance" on top of a more specific resistance that had developed in the field. It is of course still possible that some vigour tolerance is present as a result of the maintenance in the laboratory. However some of the strains have been kept in our laboratory for over two years, and during this time their resistance to parathion and diazinon remained remarkably constant.

Testing method

In most of the tests a contact method was employed. The required amounts of insecticides were dissolved in acetone to which 2 μl of peanut oil was added per ml. One ml of the solution was introduced per jar (surface 130 cm^2). The acetone was evaporated, leaving the insecticide in the peanut oil on the walls. A piece of filter paper to take up excess of moisture and a piece of cotton wool soaked with milk for food were provided. Twenty flies, males and females mixed, were kept in the jars at 25° C for 24 hours. At least four concentrations were used giving mortalities between 0 and 100% . Several replicates were made, with flies from different breeding batches. The jars were closed with cottonwool. Only in experiments with the highly volatile DDVP screw caps were used and consequently the exposure time had to be limited to 5 hours.

The method proved satisfactory for the more toxic compounds used and approximately parallel dose mortality lines were obtained. With the less toxic compounds, however, residues of more than 250 μg were needed for some of the resistant strains, and the slope of the lines decreased markedly, which may have been caused by a reduction of the percentual pick up at high dosages. No LD's$_{50}$ could be estimated in these cases, as for instance with malathion in strain G. Since it was desirable to have comparable results also with this toxicant, Busvine's method was tried besides the contact method. In doing so solutions of malathion in olive oil, 0.5 μl per fly, were topically applied to the dorsal thorax.

Insecticides

Highly purified samples of the following insecticides were used:

parathion	0,0-diethyl 0-p-nitrophenyl phosphorothionate
paraoxon	diethyl p-nitrophenyl phosphate
diazinon	0,0-diethyl 0-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothionate
0-diazinon	diethyl 2-isopropyl-4-methyl-6-pyrimidinyl phosphate
Bayer 21/199	0,0-diethyl 0-(3-chloro-4-methylumbelliferone) phosphorothionate
O-Bayer 21/199	diethyl 3-chloro-4-methylumbelliferone phosphate
gusathion	0,0-dimethyl S-(4-oxo-3H-1, 2, 3-benzotriazine-3-methyl) phosphorodithionate
O-gusathion	dimethyl S-(4-oxo-3H-1, 2 3-benzotriazine-3-methyl) phosphorodithionate
methyl-parathion	0,0-dimethyl 0-p-nitrophenyl phosphorothionate
methyl-paraoxon	dimethyl p-nitrophenyl phosphate
DDVP	dimethyl 2 : 2-dichlorovinyl phosphate
dipterex	dimethyl 1-hydroxy-2, 2, 2-trichloroethyl phosphonate
malathion	0,0-dimethyl S(1, 2-di(ethoxycarbonyl) ethyl) phosphorodithionate

RESULTS

In Fig. 1 the LD's$_{50}$ of the various compounds are given. In order to facilitate comparison of resistance levels, the points for each strain are connected by lines. The following descripton of the resistance of the strains can be given.

Strain U_2, strongly selected for γ-BHC resistance, shows a low resistance to all phosphate compounds tested. This is probably a case of vigour tolerance, caused by many non-specific physiological factors, which are also of importance in BHC resistance.

Strain A, selected with diazinon, is peculiar in having a low parathion resistance and practically none to paraoxon. In other respects its resistance resembles that of strain F and C although generally it is somewhat lower.

Strain D, selected with parathion, shows only a moderate resistance to the ethyl compounds. In other respects it resembles strain F and C.

Strain F and C, both selected with diazinon, are the ones that show the highest

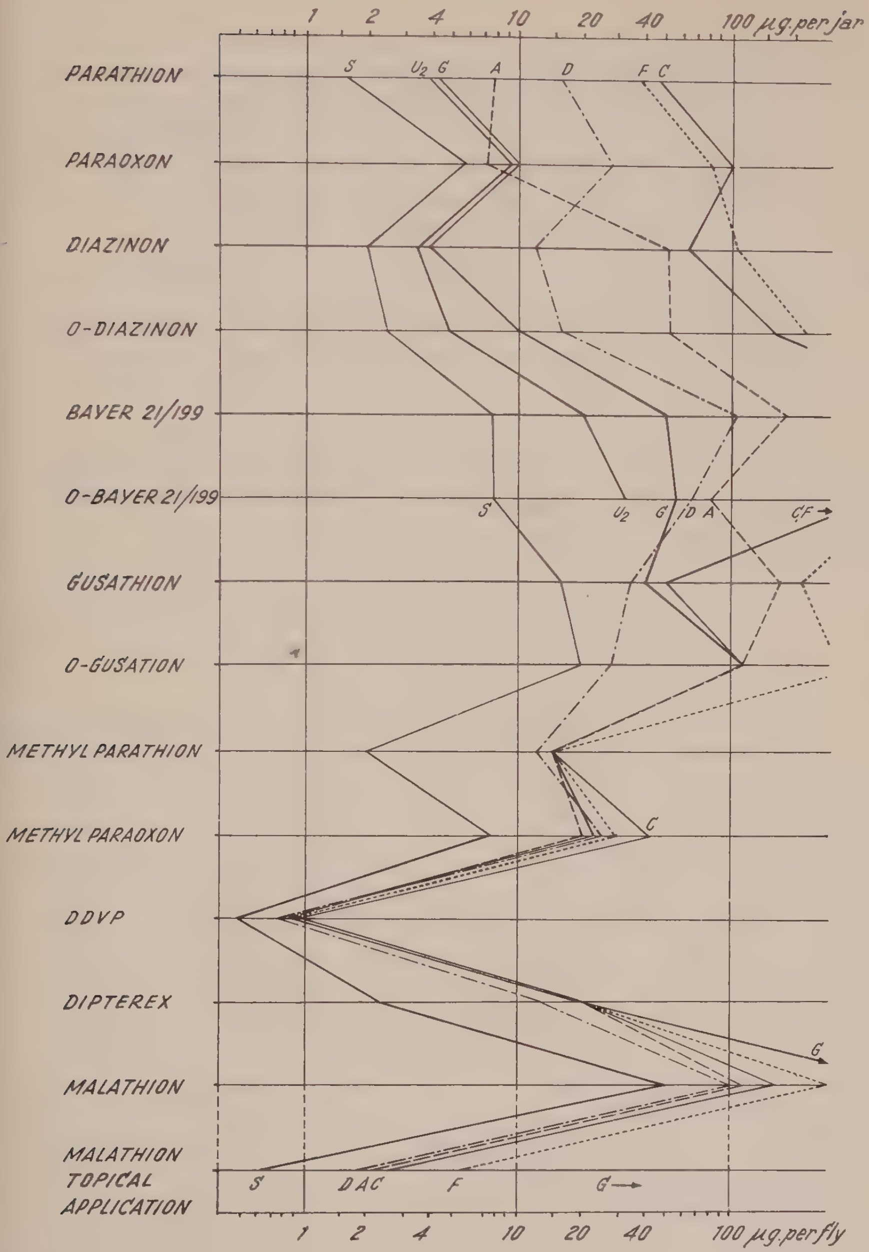

Fig. 1. Susceptibility to organophosphates in various strains of houseflies. S = susceptible; U2 = γ-BHC selected; D = parathion selected; A, C and F = diazinon selected; G = malathion selected.

resistance of all the diethyl compounds. To most of the dimethyl compounds, however, their levels of resistance are rather low. This fact will be discussed below.

Strain G, selected with malathion, has a very high resistance only to this compound. Although with topical application higher levels of resistance can be estimated than with the contact method, no LD_{50} could be estimated even with this method ($>$ 35 μg per fly, as compared with 12 μg in the American strain of Busvine).

DISCUSSION

Comparison of the methods

The resistance to malathion has been measured both with the contact method and with topical application. The results are reasonably similar. If the LD_{50} of a strain, found in the contact method is divided by 20, the number of flies per jar, a figure is obtained which can be compared with the LD_{50} per fly as found with topical application. In this way a rough estimation has been made of the percentage of toxicant actually picked up in the contact experiments. These percentages were found to be 25, 36 and 40% for strain S, D and F respectively.

Taking into account the data obtained by Busvine with strain D and C, the comparison can be extended to parathion, paraoxon, methyl parathion, diazinon and DDVP (Table I). For some compounds the LD_{50} per fly in the contact method is much higher than after topical application, but for others it appears to be equal to it or even lower. This result is very curious because it would mean that the efficiency with which the toxicant reaches the flies in the contact method can be equal to or even better than that with topical application. A probable explanation of this fact could be that the more volatile toxicants such as DDVP and diazinon reach the flies almost quantitatively, because they act in the gaseous phase as well as by contact. Non-volatile toxicants, like paraoxon, have to be

TABLE I

Comparison of toxicity of various compounds in the contact method and upon topical application.

	Strain	LD_{50} contact	$\frac{LD_{50}\text{ contact}}{20}$	LD_{50} top. appl.	$\frac{LD_{50}\text{ contact}/20}{LD_{50}\text{ top. appl.}}$
parathion	D	16	0.8	0.49 *	1.6
	C	47	2.35	1.8 *	1.3
paraoxon	D	28	1.4	0.19 *	7.4
	C	100	5	0.49 *	10.2
methyl parathion	D	12	0.6	0.22 *	2.7
	C	15	0.75	0.39 *	1.9
diazinon	D	12	0.6	0.63 *	1.0
	C	62	3.1	2.3 *	1.3
DDVP	D	0.8	0.04	0.07 *	0.6
	C	1.0	0.05	0.084 *	0.6
malathion	D	100	5	1.8	2.8
	C	60	3	2.5	1.2

* Data from BUSVINE, 1959.

picked up during the locomotory activity, and consequently much higher LD's$_{50}$ are found for them.

Differences in volatility apparently play a more important role in the contact method than with topical application. In spite of these differences between the methods it appears that the levels of resistance found, as far as they could be compared, are not much different.

Resistance to dimethyl compounds

Busvine (1959) has pointed out that the degrees of resistance of strains C and D widely differ for the ethyl compounds, but much less for the methyl compounds, with the exception of dicapthon.

TABLE II

Comparison of ratio's of LD's$_{50}$ of diethyl and dimethyl compounds.

	LD$_{50}$				Ratio LD$_{50}$		
strain:	C	F	A	D	$\frac{C}{D}$	$\frac{E}{D}$	$\frac{A}{D}$
parathion	46	38	7.7	16	2.9	2.4	0.5
paraoxon	100	82	7	28	3.6	2.9	0.3
diazinon	62	105	52	12	5.2	8.8	4.3
O-diazinon	160	225	52	16	10	14	3.3
Bayer 21/199	>250	>250	180	105	—	—	1.7
O-Bayer 21/199	>250	>250	82	63	—	—	1.3
gusathion	51	220	170	34	1.5	6.5	5.0
O-gusathion	115	>250	115	28	4.1	—	4.1
methyl parathion	15	15	15	12	1.3	1.3	1.3
methyl paraoxon	41	28	20	25	1.6	1.1	0.8
DDVP	1	0.9	0.9	0.8	1.3	1.1	1.1
dipterex	20	20	20	12.5	1.6	1.6	1.6
malathion	160	280	115	100	1.6	2.8	1.1

In Table II the LD's$_{50}$ of strain C, F and A are compared with that of strain D. The relation observed by Busvine is found in strain C and F, except for gusathion and its oxygen analogue, and with malathion as a borderline case. It is not found in strain A. The phenomenon may be explained by assuming that resistance to the ethyl-compounds is brought about mainly by rather specific mechanisms, and to a lesser extent by some less specific mechanisms giving also resistance to the methyl compounds.

Control of resistant flies

It is notable in Fig. 1 that the degree of resistance to many of the methyl compounds, especially DDVP and dipterex is comparatively low. It must be borne in mind, however, that a strain with high dipterex resistance has been found (Labrecque et al., 1958) and since this toxicant is thought to act via its conversion into DDVP (Metcalf, Fukuto & Marsh, 1959), it would not be surprising that DDVP resistance will be also present in these strains. However, no serious resistance to DDVP has been reported so far. Since DDVP has a very low toxicity to mammals it may be the most favourable compound for fly control tested in this study.

Number of resistance mechanisms

From the fact that every strain has its own pattern of resistance it can be concluded that a relatively large number of resistance mechanisms must be involved. The patterns of strain F and C are the only two that have much in common. This large number of possibilities is serious, since it suggests that still other mechanisms may be selected out, that cause resistance to any new member of this group of insecticides.

Whereas it seems improbable that strain G would have important resistance genes in common with the other strains, it may be that this is the case with strains D, A, F. and C. E. g. the low resistance found in strain D could be caused by part of the factors present in strain F. An interesting case is the presence of paraoxon resistance in strain F, and its absence in strain A. The paraoxon resistance of strain F seems to be true cross-resistance since it was developed by selection with diazinon only. This was confirmed by the following experiment: strain F was crossed with strain S and the progeny was alternatively selected with diazinon and backcrossed with strain S. It was found that the paraoxon resistance was indeed maintained by selection with diazinon (scheme of the crosses in *Ent. exp. & appl.* 2, p. 50).

In a previous communication (VAN ASPEREN & OPPENOORTH, 1959) it was shown that five strains resistant to phosphate insecticides have a low ali-esterase activity to certain esters, whereas six susceptible strains have high activities. It was concluded that the gene(s) for low ali-esterase must be responsible for at least part of the resistance of the strains. The most simple situation would be that the same gene(s) for low ali-esterase were present in all resistant strains. Differences between the strains must then be caused by other genes. It will be apparent from Fig. 1 that if this situation was true, the gene(s) for low ali-esterase could be only responsible for the very small part of the total resistance which all strains have in common. E.g. the low malathion resistance of strain D or the low diazinon resistance of strain G would be caused by this gene. It is, however, unlikely that this low resistance would have been selected out in all strains. There are two alternative possibilities. First every strain could have its own gene(s) for low esterase activity, connected with a special type of resistance. Second it could be that one common gene for low ali-esterase activity is present in all strains, which only by cooperation with other genes confers the specific resistance mechanisms of the strains.

Nature of resistance mechanism

In most cases resistance to the oxygen analogues is of the same order of magnitude as to the thio compounds. The presence of a resistance mechanism based on a decreased conversion to the oxygen compound is, therefore, less probable. Since it is known that much less paraoxon is present after intoxication with parathion in the resistant strain D than in strain S (OPPENOORTH, 1958a) it is most probable that a detoxication mechanism is present which acts either on paraoxon only, or on paraoxon and parathion.

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ZUSAMMENFASSUNG

FORMEN DER RESISTENZ GEGENÜBER ORGANISCHEN PHOSPHORVERBINDUNGEN BEI EINIGEN STUBENFLIEGEN-STÄMMEN

Die Empfindlichkeit eines normal-sensiblen und von sechs resistenten Stubenfliegen-Stämmen gegenüber 13 organischen Phosphorverbindungen wurde mit Hilfe einer Kontakt-Methode gemessen. Fünf dieser Stämme waren sowohl im Freiem wie auch im Labor, mit Diazinon, Parathion oder Malathion selektiert worden. Ein Stamm war stark mit γ-BHC selektiert worden. Die Empfindlichkeit der Stämme ist in Fig. 1 dargestellt. Der mit γ-BHC selektierte Stamm wies nur eine niedrige Resistenz gegenüber den organischen Phosphorverbindungen auf. Höhere Resistenz wurde in den mit organischen Phosphorverbindungen selektierten Stämmen gefunden; jeder dieser Stämmen zeigte eine eigene, charakteristische Form der Resistenz. Dies deutet darauf hin, daß eine relativ grössere Zahl von Resistenzmechanismen vorhanden ist.

Aus der Tatsache, daß Resistenz gegen die Thioverbindungen und ihre entsprechenden Sauerstoff-Analoge ungefähr gleich groß ist, wird geschlossen, daß eine langsamere Umwandlung in die Phosphate als Resistenz-mechanismus nicht in Betracht kommen kann.

Die Resistenz gegenüber einigen Methyl-verbindungen ist gering, und in den einzelnen Stämmen nur wenig verschieden. Diese Resistenz wird wahrscheinlich durch weniger spezifisische Faktoren verursacht. Die genetische Grundlage des niedrigen Ali-esterasen-gehalts in den Phosphat-resistenten Stämmen wird diskutiert.

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SOME FACTORS INFLUENCING THE EMERGENCE OF OVERWINTERING HESSIAN FLY LARVAE

BY

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Overwintering fully-fed Hessian Fly larvae in puparia respond to experimental conditions of constant temperature and relative humidity progressively faster as the normal time of pupation and emergence in the spring approaches. Frost does not speed up emergence. A constant temperature of 15° or 20° C at 95% RH is recommended for the production of midges for experimental purposes from overwintering Hessian Fly puparia.

After the Hessian Fly (*Mayetiola destructor* Say) larva has finished feeding it undergoes a final larval ecdysis within a hard brown puparium. Most larvae derived from eggs laid between April and July continue to develop and pupal formation is succeeded by midges emerging. But some individual larvae from such eggs do not turn into pupae for a long time, even until the following year. As the date of egg-laying approaches the autumn an increasing proportion of larvae behave in this manner until a date is reached after which development is not completed by any individual until the next year. In an untreated and open glasshouse at Harpenden, this date is September 1. Eggs laid after this have not been known to develop into midges the same year although emergence of at least some individuals from eggs laid up to the end of August has continued until early November (BARNES, 1958).

There is no precise information about the continuance of development after larval feeding stops. MILLER & JOHNSON[1]) obtained data on near-optimum temperature and humidity conditions required for emergence but the stock of puparia used had been stored in a refrigerator for several weeks at 5° C and its history was rather uncertain.

The present investigations had two purposes: to obtain information on (1) the factors stimulating pupation, and finally emergence of midges, in fully-fed overwintering larvae in puparia and (2) on means whereby a constant supply of adults can be maintained for experimental purposes.

MATERIAL

The puparia needed for the experiments were obtained from Hessian Fly of English, German and U.S.A. origin that had been interbred at Rothamsted for 1½ years. The exact intermixture of parentage is known but is unnecessary here.

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[1]) MILLER, B. S. & JOHNSON, J. A., Final Report on National Science Foundation Grant No. G-1299. A study of the biochemical factors in the resistance of the wheat plant to attack by the Hessian Fly. January 6, 1958 (Unpublished).

The midges were reared on Peko wheat in flats or seed-boxes, 8½ × 14 × 3 inches, in an open glasshouse. The seed rate was approximately 1 seed per ½ inch in 8 rows one inch apart. This required about 200 seeds, roughly 20 g per flat.

The wheat was sown in late August 1958. Each flat was immediately covered with a muslin cage which was retained in position throughout the breeding. This is an important precaution for, although it encourages the growth of mildew on the plants, extraneous parasites are liable to infest the developing larvae if the cages are removed after the parent midges have finished ovipositing. The mildew does not appear to affect the satisfactory development of the midges. The parent Hessian Flies (12—20 females with 7—16 males per flat) were put in the cages early in September, because oviposition before September 1 under these open glasshouse conditions results in the filial generation starting to emerge before the winter.

At the end of October and early in November the puparia were extracted. Ten flats produced 12,285 puparia in 1,321 untillered plants, an average of slightly over 9 per stem. Details are given in Table I.

TABLE I

The breeding of Hessian Fly on Peko and Tenmarq wheat, 1958

Flat	Sowing date	No. of ♀♀ put in	Date	No. of puparia extracted	Date	No. of plants	Av. no. of puparia per plant
P1	Aug. 25	12	Sept. 6	1093	Oct. 30-31	138	7.9
P2	"	12	"	1237	"	113	10.9
P3	"	12	"	1090	"	119	9.2
P4	"	12	"	916	Oct. 30-Nov. 3	128	7.2
P5	Aug. 27	15	Sept. 7	1286	"	126	10.2
P6	"	14	"	968	"	130	7.4
P7	"	15	"	1346	Nov. 3-5	127	10.6
P8	"	15	"	1301	Nov. 3-6	145	9.0
P9	Aug. 29	20	Sept. 8	1864	Nov. 3-5	134	13.9
P10	"	15	"	—	—	—	—
P11	"	18	"	—	—	—	—
P12	"	18	"	1184	Nov. 5-7	161	7.4
T1	Aug. 15	20	Aug. 30	2002	Nov. 27-Dec. 3	143	14.0
T2	"	20	"	—	—	—	—
T4	Aug. 18	15	Sept. 2	1482	Nov. 27-Dec. 3	149	10.0
T5	"	15	"	1819	Dec. 3-Jan. 5	163	11.2
T7	Aug. 21	10	Sept. 5	1288	Dec. 3-4	166	7.8
T8	"	10	"	1271	Jan. 7-8	162	7.8

Tenmarq wheat grown under similar conditions provided a further supply of puparia. But as the leaves had been cut off for biochemical analyses on September 25 before all the larvae had completed their feeding, the proportion of small puparia was greater than with the Peko wheat. Furthermore, to control mildew the plants were not caged after early September and consequently some larvae became parasitised. The puparia were removed from the stems later than those from Peko.

Details of this breeding are included in Table I. Five flats produced 7,862 puparia in 783 untillered plants, an average of 10 per stem.

After extraction the puparia were kept moist in petri dishes in the insectary until required.

METHODS

Glass-topped seven-pound Kilner jars were used to keep puparia in specified relative humidities. Crystallizing dishes (90 mm in diameter and 50 mm high) containing 125 ml of potassium hydroxide solution were placed inside these jars. The lips of the dishes were coated with paraffin wax to prevent creeping of the caustic solution, made as described by BUXTON & MELLANBY (1934) to keep the relative humidity at 95, 85 and 75%. Tests after the experiments ended showed that the relative humidity had remained within 1% of the original. A fluffy mass of glass wool placed in the caustic solution prevented excessive movement of the liquid while the jars were being moved.

Constant temperatures were obtained by placing the Kilner jars either in one of the constant temperature rooms that were maintained at 0°, 5°, 10° and 20° C or in one of the cabinets maintained at 15°, 25°, 30° and 35° C. The rooms varied only ± 0.1°, but the cabinet at 15° varied ± 1° and those at 25°, 30° and 35° varied ± 2°.

Hessian Fly puparia in units of 100 were placed in glass tubes (25 mm in diameter and 76 mm high) which were covered with a muslin cap held in shape with sticky tape. Usually multiples of three such units were used in each experiment. The tubes were arranged around the crystallizing dish in the bottom of the Kilner jars. The midges and parasites which emerged were collected daily beginning at about 11.00 hours. Later they were counted and the sex determined. The Kilner jars and tubes were out of the constant temperatures only for so long as it took to remove the insects, usually a minute or so daily. The changes of temperature may be of importance, but only apply in those experiments where cabinets, not rooms, were used.

EXPERIMENTS

The experiments were designed to study the effect of various constant temperatures and relative humidities on the further development of autumn-fed larvae in puparia that would not normally complete their development until the spring.

The main experiment (1) was to discover if there are any differences in effect on such larvae that had been exposed A. for a short time to ordinary autumnal fluctuating temperatures (i.e. during October) without being frosted and B. for a long period to autumnal and winter fluctuating temperatures (i.e. during October to January inclusive) including being subjected to ones below 0° C. The puparia of these two types are referred to from now on as Material A and B.

The second experiment (2) was planned to reveal the effect after exposure to ordinary fluctuating temperatures for varying periods. This was done by using puparia from the insectary at monthly intervals throughout the autumn and winter up to the time emergence usually starts in the spring.

In another experiment (3) puparia, that had not been frosted naturally, were subjected for varying periods (32, 60 and 90 days) to various constant low temperatures (10°, 5°, 0° and —6°) before being placed in a higher temperature (20°).

In the fourth experiment (4) the effect of light on puparia that had not been frosted was examined.

Supplementary experiments were made on the effect of keeping puparia at 10°, 5° and 0°, as well as the effect of spraying the puparia with a fungicide in order to keep down the mould that was liable to develop at the higher relative humidities. The mould however does not seem to have any deleterious effect on the emergence of the midges.

RESULTS

Experiment 1

Peko-reared larvae in puparia were used in both halves of the experiment: the half with Material A (see above) was started on November 4, and that with Material B on February 4.

TABLE II

Emergence at 15° C of Material A and B

% RH	Replicate	Number of days to 1st emergence	50% emergence	Duration of emergence in days	No. of midges ♂	♀	total
A 75	1	17	32	51	25	55	80
	2	16	30	33*	24	56	80
	3	17	30	58	28	59	87
85	1	17	28	51	28	55	83
	2	17	28	50	30	60	90
	3	18	28	51	32	56	88
95	1	15	28	50	32	55	87
	2	14	30	43	31	57	88
	3	17	30	47	22	69	91
B 75	1	14	17	14	28	66	94
	2	14	16	9	42	54	96
	3	14	17	10	32	64	96
85	1	15	17	12	38	62	100
	2	14	17	12	41	52	93
	3	15	17	12	37	55	92
95	1	13	17	14	36	59	95
	2	14	17	9	38	58	96
	3	14	17	9	38	59	97

* the potassium hydroxide solution leaked into this tube.

At 15° C emergence was good at 75, 85 and 95% RH (Table II). Material B started to emerge, reached the 50% mark and completed emergence quicker and gave a greater percentage emergence than Material A.

TABLE III

Emergence at 20° C of Material A and B

	% RH	Replicate	Number of days to 1st emergence	Number of days to 50% emergence	Duration of emergence in days	No. of midges ♂	No. of midges ♀	No. of midges total
A	75	1	13	21	24	17	35	52
		2	13	21	21	19	46	65
		3	15	20	19	16	39	55
	85	1	14	21	27	21	42	63
		2	12	20	21	18	50	68
		3	12	20	29	22	57	79
	95	1	13	19	16	29	48	77
		2	13	19	15	20	64	84
		3	12	19	20	28	49	77
B	75	1	10	12	7	28	66	94
		2	9	11	8	42	54	96
		3	10	11	6	35	57	92
	85	1	10	11	6	36	62	98
		2	10	11	9	36	62	98
		3	10	11	6	33	66	99
	95	1	9	11	5	36	59	95
		2	9	11	7	42	56	98
		3	9	11	9	38	55	93

At 20° C emergence at all three levels of relative humidity (Table III) was again started and completed quicker by Material B than A. A considerably greater percentage of midges emerged from Material B than A. Emergence from Material A and Material B was quicker than at 15°: fewer midges emerged from A, but as many from B as at 15° C.

At 25° C (Table IV) emergence from Material A was small, improving slightly the higher the relative humidity. On transference to 15° more midges emerged though somewhat slowly. Emergence was much greater from Material B although some midges able to emerge as pupae from the puparia could not break out from their pupal cases.

Emergence of both sets of material was less satisfactory at 25° than either at 15° or 20°. Furthermore mould developed more freely in the tubes and, although this did not appear to affect the emergence, the newly emerged midges got entangled in it.

TABLE IV

Emergence of Material A (i) at 25° C, (ii) on transference to 15° and of Material B at 25°

% RH	Replicate	Number of days to		Duration of emergence in days	No. of midges		
		1st emergence	50% emergence		♂	♀	total
A (i) 75	1	—	—	—	nil	nil	nil
	2	9	11	5	2	1	3
	3	11	13	5	1	2	3
85	1	9	13	8	4	14	18
	2	8	13	9	4	9	13
	3	9	14	13	6	5	11
95	1	10	14	17	14	20	34
	2	10	14	19	12	28	40
	3	8	14	20	18	22	40
A (ii) 75	1	18	22	21	24	50	74
	2	17	22	25	25	56	81
	3	18	22	22	26	44	70
85	1	17	21	27	15	48	63
	2	17	22	19	27	43	70
	3	17	22	22	28	47	75
95	1	16	19	14	17	30	47
	2	19	22	15	11	30	41
	3	18	21	13	18	27	45
B 75	1	8	10	10	52	22	81*
	2	8	10	13	56	31	90*
	3	8	10	10	58	29	91*
85	1	8	10	12	48	32	88*
	2	8	9	7	54	20	90*
	3	8	10	12	50	34	91*
95	1	8	9	6	32	53	92*
	2	8	9	6	35	53	89*
	3	8	9	5	31	59	96*

* the totals include pupae that although they emerged from puparia did not complete emergence into midges.

At 30° C Material A failed entirely to emerge, although some midges emerged at each humidity after transference to 20° (Table V). By comparison Material B produced pupae and partially emerged midges from the puparia at each humidity and midges emerged successfully after transference to 20° (Table VI), although rather slowly.

Emergence from both sets of material was therefore less at this temperature than at 15°, 20° and even 25°. Some puparia seemed to be killed at 30° C.

TABLE V

Emergence of Material A at 20° C after 1 month's exposure to 30° C

% RH	Replicate	Number of days to 1st emergence	Number of days to 50% emergence	Duration of emergence in days	No. of midges ♂	No. of midges ♀	No. of midges total
75	1	13	34	69	25	37	62
	2	13	34	73	23	28	52*
	3	14	33	70	16	34	51*
85	1	13	27	61	29	44	73
	2	13	26	63	31	48	79
	3	12	19	70	24	59	83
95	1	11	7	60	37	59	96
	2	11	6	55	31	57	88
	3	11	15	54	24	23	47

* includes pupae that emerged from the puparia but from which the midges did not emerge.

TABLE VI

Emergence of Material B (i) during 35 days' exposure to 30° C and (ii) on transference to 20° C

	% RH	Replicate	Number of days to 1st emergence	Number of days to 50% emergence	Duration of emergence in days	No. of midges ♂	No. of midges ♀	No. of midges total
(i)	75	1	10	11	4	—	—	19*
		2	10	11	3	—	—	18*
		3	10	11	4	—	—	24*
	85	1	11	11	3	—	—	8*
		2	11	12	2	—	—	7*
		3	11	11	1	—	—	3*
	95	1	10	11	5	—	—	26*
		2	10	11	4	—	—	20*
		3	11	11	3	—	—	9*
(ii)	75	1	29	33	16	4	7	11
		2	15	31	43	3	2	5
		3	29	40	27	4	6	10
	85	1	15	30	53	9	24	33
		2	12	29	43	12	16	28
		3	14	28	46	13	16	29
	95	1	13	24	38	12	18	30
		2	12	17	40	12	13	25
		3	12	19	33	12	22	34

* Pupae and partially emerged midges.

At 35° C there was no emergence from either Material A or B at any of the three relative humidities. On transference to 20° and again later to 15° some emergence of Material A took place in 85 and 95% RH (Table VII) but none occurred in 75% RH. Material B was not transferred to a lower temperature. There is evidence of a higher mortality at this temperature than at 30° C.

TABLE VII

Emergence of Material A after 1 month's exposure to 35° C, at (i) 20° and then (ii) 15°

	% RH	Replicate	Number of days to 1st emergence	Number of days to 50% emergence	Duration of emergence in days	No. of midges ♂	No. of midges ♀	No. of midges total
(i)	85	1	—	—	—	nil	nil	—
		2	28	47	21	0	5	5
		3	20	30	40	6	17	23
	95	1	21	29	34	12	16	28
		2	16	28	41	11	22	33
		3	15	22	48	16	33	49
(ii)	85	1	—	—	—	nil	nil	—
		2	6	20	15	2	3	5
		3	6	17	23	2	14	16
	95	1	1	17	21	1	11	12
		2	8	18	20	1	18	19
		3	3	18	28	2	23	26*

* includes 1 pupa that emerged from the puparium but from which the midge did not emerge.

The above show that in the constant temperatures and relative humidities tested emergence was quicker and better from Material B than Material A. In other words overwintering larvae in puparia that have been kept in near-outside fluctuating temperatures for a short time and without frost react more slowly and less than do those that have been exposed for a long time to the fluctuating temperatures including frost.

This suggests that the fully-fed overwintering larvae in puparia develop slowly throughout the winter. Experiment 2 was designed to test this hypothesis.

Experiment 2

A constant temperature of 20° C and relative humidity of 95% were employed throughout this experiment, in which larvae reared on Peko wheat and on Tenmarq were used at approximately monthly intervals throughout the winter. The results are given in Table VIII and IX. The longer the larvae in puparia had been kept in the insectary at fluctuating temperatures, i.e. the nearer the normal time for pupation and emergence in the spring, the quicker emergence started, reached 50% and was completed. This supports the hypothesis that development of the fully-fed overwintering larvae in puparia continues throughout the winter. Parasites appeared from the Tenmarq-reared larvae, no doubt because the cages had been removed soon after egg-laying had been completed (see p. 225). The Tenmarq-reared larvae usually gave a significantly lower percentage emergence than did the Peko-reared larvae, probably because the larvae had less food (see p. 225).

TABLE VIII

Peko-reared larvae. Emergence at 20° C 95% RH at intervals through the period of non-emergence in the field

Date expt. started	Replicate	Number of days to 1st emergence	Number of days to 50% emergence	Duration of emergence in days	No. of midges ♂	♀	total
4 Nov.	1	13	19	16	29	48	77
	2	13	19	15	20	64	84
	3	12	19	20	28	49	77
14 Nov.	1	11	14	13	67	22	89
4 Jan.	1	10	11	5	71	26	97
	2	10	11	4	70	24	94
	3	10	11	4	68	26	94
4 Feb.	1	9	11	5	36	59	95
	2	9	11	7	42	56	98
	3	9	11	9	38	55	93
4 Mar.	1	8	10	6	63	33	96
	2	9	10	7	63	27	90
	3	7	10	6	51	40	91
	4	8	10	7	38	54	92
4 Apr.	1	4	7	10	61	34	95
	2	4	7	8	52	39	91
	3	3	7	9	45	49	94
	4	4	7	8	55	40	95

TABLE IX

Tenmarq-reared larvae. Emergence at 20° C 95% RH at intervals through the period of non-emergence in the field

Date expt. started	Replicate	Number of days to 1st emergence	Number of days to 50% emergence	Duration of emergence in days^{+}	No. of midges and parasites ♂	♀	p	total
4 Dec.	1	14	17	13(13)	24	53	3	80
	2	14	18	17(17)	24	55	5	84
	3	14	18	12(12)	23	44	5	72
4 Feb.	1	10	13	20(20)	14	58	6	78
	2	10	13	14(14)	23	55	1	79
	3	10	13	16(20)	21	52	5	79*
4 Mar.	1	8	10	7(10)	27	60	2	89
	2	9	10	5(11)	25	48	2	75
	3	8	10	7(11)	15	68	2	85
	4	8	11	9(13)	20	50	10	80
4 Apr.	1	4	7	8(15)	23	35	7	65
	2	4	8	7(19)	23	50	4	77
	3	3	7	8(14)	25	51	3	79
	4	4	7	10(19)	21	51	3	75

* includes 1 pupa that emerged from the puparium but from which the midge did not emerge.

$^{+}$ figures without brackets indicate the duration of emergence of the midges only.

Experiment 3

To test whether constant low temperatures increase the rate of larval development more than do fluctuating temperatures (see Experiment 2), Tenmarq-reared larvae at the beginning of December, i.e. before they had been exposed to frost, were placed at 85% RH in constant temperatures of 10°, 5°, 0° and —6° C for 32, 60 and 90 days. Then they were placed at 95% RH in a constant temperature of 20° C. There is no evidence from Table X that emergence took place quicker after frosting. Midges emerged in both the tubes of puparia that had been at 10°

TABLE X

Emergence of Tenmarq-reared larvae at 20° C 95% RH after pretreatment at various low temperatures lasting (i) 32 days, (ii) 60 days and (iii) 90 days

Pretreatment temp. ° C.	Replicate	Number of days to 1st emergence	Number of days to 50% emergence	Duration of emergence in days+	No. of midges and parasites ♂	♀	p	total
10	1	11	13	15(24)	4	63	8	75
	2	11	13	10(12)	4	63	4	71
5	1	11	14	14(14)	2	50	4	56
	2	11	14	15(15)	3	60	5	68
0	1	11	14	22(22)	7	58	2	67
	2	12	14	18(18)	10	57	3	70
—6	1	11	15	16(16)	5	48	0	53
	2	11	15	22(22)	8	36	4	48
10	1	8	12	14(15)	3	74	4	82*
	2	8	12	8(21)	3	72	4	79
5	1	11	13	6(12)	9	60	2	71
	2	11	13	8(11)	5	67	1	74*
0	1	11	13	12(12)	9	60	1	70
	2	11	13	13(16)	7	55	3	65
—6	1	10	13	21(21)	7	55	2	64
	2	11	13	16(21)	5	55	2	62
10	1	1	6	13(30)	11	62	6	79
	2†	1	—	—	—	—	—	—
5	1	10	12	7(18)	6	70	2	78
	2	10	12	10(15)	25	52	6	83
0	1	8	10	9(12)	8	64	1	73
	2	8	10	6(22)	11	62	2	75
—6	1	10	13	20(20)	8	68	2	78
	2	10	13	12(13)	9	70	1	80

+ Figures without brackets indicate the duration of emergence of the midges only.

* Includes pupae that emerged from the puparium but from which the midges did not emerge.

† See also Table XII.

for 90 days after one day at 20° 95% RH. One of these tubes was therefore replaced in 10° (see later on, Supplementary Experiment 1).

It was observed that, although the tubes of puparia were allowed to remain at 20° 95% RH for at least fourteen days after the last midge or parasite had emerged, a few midges emerged during the six weeks the tubes were back at ordinary room temperature. Thus 10 additional midges emerged at room temperature and humidity in the tube that had received 30 days' pretreatment at 0°. One midge emerged in the tube that had received 60 days' pretreatment at 0° and 1 and 4 midges respectively emerged in the tubes that had received 60 days' pretreatment at—6°, these tubes were kept at room temperature as well as humidity. One midge emerged in each tube that had received 90 days' pretreatment at 0° and 5 and 2 midges in those that had had 90 days' pretreatment at —6°, these tubes were retained at 95% RH while at room temperature. No midges emerged after pretreatment at 10° or 5°.

Experiment 4

This experiment was started on January 12, i.e. before the larvae in puparia had been subject to frost. The amount of light (day-length) the puparia received did not affect the speed or extent of emergence, although the emergence of the midges was completed quicker when more light was given. The lighting, between 75 and 225 foot candles, was by reflection from warm white fluorescent tubes. The results are given in Table XI. Since the puparia in this experiment were kept at 20° C and 95% RH, the results can be used as additional evidence for the emergence of Tenmarq-reared larvae at intervals through the period of non-emergence in the field (see Table IX), filling in the gap between December 4 and February 4.

TABLE XI

Emergence of Tenmarq-reared larvae at 20° C 95% RH during different amounts of light

Hours of light	Replicate	Number of days to		Duration of emergence in days*	No. of midges			
		1st emergence	50% emergence		♂	♀	p	total
0	1	10	12	15(15)	23	59	2	84
	2	10	13	18(18)	35	47	4	86
	3	10	12	13(13)	21	56	2	79
8	1	9	12	12(12)	26	57	4	87
	2	9	12	15(15)	16	69	0	85
	3	9	12	13(15)	25	57	6	88
16	1	10	12	11(17)	25	59	2	86
	2	10	12	11(16)	27	62	2	91
	3	10	13	9(13)	23	58	5	86
24	1	9	12	8(12)	29	53	3	85
	2	9	11	8(11)	24	60	4	88
	3	9	12	8(14)	31	52	2	85

* Figures without brackets indicate the duration of emergence of the midges only.

Supplementary Experiment 1

The fact that midges from puparia previously kept for 90 days at 10° C emerged the day after they had been placed at 20° C 95% RH (see Experiment 3, Table X), indicated that the overwintering larvae must have developed further at 10°. This was not unexpected because of the successful emergence at 15° (see Experiment 1). As already stated, one of these units (1 tube containing 100 puparia) was replaced at 10° and at the lower relative humidity, 85%. In addition, two units of Peko-reared puparia that had been kept at fluctuating temperatures in the insectary since they had been removed from the wheat during late October and early November, as well as two units of Tenmarq-reared puparia that had been kept at 0° C 85% RH since December 4, were added on March 4 to the Kilner jar at 10° C 85% RH. Table XII shows that the Tenmarq-reared larvae that had been at 10° C 85% RH since December (except for one day at 20° C 95% RH) took longer to develop into midges than the Tenmarq-reared larvae that had been stored at 0° C 85% RH since December 4 and appreciably longer than the Peko-reared larvae that had been kept at fluctuating temperatures since late October—early November.

To see if midges would emerge below 10° C, two units of Tenmarq-reared larvae that had been stored at 0° C 85% RH since December 4 were placed at 5° C 85% RH on March 4. Up to June 22, i.e. 110 days, no midges had emerged.

Finally no midges had emerged by June 22 in two units of similar material that had been stored at 0° C 85 RH since December 4.

TABLE XII

Emergence of Peko- and Tenmarq-reared larvae at 10° C 85% RH

Units*		Number of days to 1st emergence	50% emergence	Duration of emergence in days	No. of midges and parasites ♂	♀	p	total[2]
Tenmarq-reared ex Expt.	3	91[1]	115	77	14	49	0	66
Peko-reared	1	48	23	106	24	21	—	62
,,	2	48	20	106	13	36	—	59
Tenmarq-reared	1	81	103	(29)[3]	6	13	0	19
,,	2	77	100	(33)[3]	1	14	0	15

* for details of pretreatment see text.

[1] 3 midges emerged the day this unit was placed at 20° C 95 RH and 1 more one day after its return to 10° C 85% RH, but then six days intervened before regular emergence at 10° started.

[2] includes pupae that emerged from puparia but from which the midges did not emerge.

[3] midges were still emerging when the experiment was stopped on June 22.

Supplementary Experiment 2

Two attempts were made to discover whether Dowicide (orthophenylphenate) at 100 mg/20 ml (H_2O), fungicide, affected emergence (see p. 227). Dowicide was first sprayed into the tubes containing the puparia. Only 2 and 10% of the midges subsequently emerged at 25° C 95% RH. In the second test the puparia were sprayed on muslin, so the excess spray could run off. The puparia were replaced in the tubes and placed at 20° C 85% RH. Subsequently 94% and 68% of the midges emerged.

EMERGENCE IN THE OPEN GLASSHOUSE AND INSECTARY

After puparia to be used in the experiments had been extracted, the flats were replaced in the open glasshouse for the winter. In the spring observations were made daily to establish the emergence numbers and dates under these conditions of fluctuating temperatures. Emergence started on April 4, a female appearing in T1. On April 6 flats P1-5, T1, T2 and T4 were moved to the insectary and the dates of emergence in the two situations compared. They showed no significant difference probably because of the late date at which the flats were moved, since previous studies have shown that emergence occurs about ten days earlier in the glasshouse than in the insectary, where emergence occurs almost at the same dates as in the open. Observations on the present emergences ceased on May 8 except in P10 and P11 where they were continued until May 31. Table XIII shows that about 900 midges emerged in each of the two Peko flats from which the puparia had not been extracted. This figure would have been close to 1,100 had it contained the puparia on the twenty stems given away from each of these flats. The level of emergence under these open glasshouse conditions approaches that obtained in the experiments where temperature and humidity were controlled. The low emergence in T2 is because most of the wheat stems had been removed for another purpose.

A few midges emerged in each of the flats from which the puparia had been removed. This suggests that some had fallen off the plants and also that it is impossible to be sure of removing 100%. No attempts were made to assess the number of parasites emerging in the Tenmarq flats.

TABLE XIII

The emergence of overwintering Hessian Fly puparia from Peko and Tenmarq wheat, 1959

Flat	Number of Midges			Dates
	♂	♀	total	
P1	0	2	2	April 10 and 25
P2	1	14	15	April 17—29
P3	0	5	5	April 22—May 4
P4	0	1	1	May 4
P5	3	2	5	April 23—May 7
T1	1	0	1	April 22
T2	4	68	72	April 15—May 7
T4	0	2	2	April 22 and 23
P6	19	5	24	April 16—May 7
P7	0	2	2	April 21 and May 7
P8	6	2	8	April 20—28
P9	1	2	3	April 14, 22 and 29
P10	353	563	916	April 13—May 4
P11	418	471	889	April 12—May 13
P12	12	5	17	April 16—May 7
T5	2	13	15	April 12—26
T7	1	5	6	April 10—25
T8	2	4	6	April 10—20

It is interesting to note that 2 and 8 female midges had previously emerged on October 14 and between October 14 and 25 respectively in flats T1 and T2. This and the lack of emergences during the autumn in the other flats (see Table I) confirm the observation that oviposition before September 1 results in a partial emergence before the winter.

As has already been stated, the puparia that had been removed from the plants were stored in petri dishes in the insectary, thus being exposed to fluctuating temperatures. From March 5 onwards 10 puparia from both the Peko- and Tenmarq-reared puparia were opened at intervals to trace the pupation and emergence under these near-normal conditions. Table XIV gives the results.

TABLE XIV

Development under insectary conditions of larvae into pupae within the puparia

Date		Peko-reared		Tenmarq-reared	
		Larvae	Pupae	Larvae	Pupae
March	5	10	0	10	0
,,	19	7	3	8	2
,,	26	5	5	8	2
April	2	3	7	7	3
,,	10	2	8	3	7
,,	16	0	10	3	7

Midges first appeared among the puparia extracted from Peko wheat on April 16 and from those reared on Tenmarq on April 21. Under these conditions pupation started in 1959 in the latter part of March, and emergence in the second half of April.

DISCUSSION

When comparing the results of these experiments with others of similar experiments on other insects it must be realized that (i) the puparia were all produced over a limited period but their exact age was not known when they were removed from the wheat, (ii) under natural conditions none of the puparia would have given rise to midges until the following spring, i.e. about 220 days, and (iii) the periods measured in these experiments covered two stages in development (a) up to the time the larvae pupated and (b) the pupal period. It is (a) that varies more in length and was more affected by the experimental conditions. It can be postulated that (b) as in most insects is affected in a simple way by temperature and that above the "development zero" its duration is determined fairly accurately by temperature.

A high percentage emergence was obtained at 15° C from young and unfrosted puparia as well as from older frosted puparia, but at 20° and 25° a higher percentage was obtained from the older frosted puparia. At 30° the younger unfrosted puparia failed to emerge although pupae in the older frosted material were stimulated to come out of the puparia. The midges emerged successfully after transference to 20°. At 35° no emergence occurred although even this high temperature did not kill all the larvae as some midges emerged when the puparia were removed to a lower temperature. At the opposite extreme, emergence was slight at 10°, and failed below 5°.

The speed of development at 20° increased gradually as the spring approached. For example, puparia placed at this temperature in early November took 12—13 days to start emergence whereas others placed at 20° in early April took only 3—4 days. The time for 50 per cent emergence was also shortened.

Relative humidity (75, 85 and 95%) had little or no effect on the period of development at 15°. At 20°, 75% RH seemed to decrease emergence, but this was probably only delayed emergence, for after a similar reduction at 25°, both in 75 and 85% RH, full emergence was obtained by lowering the temperature to 15°. At 35°, however, the relative humidity may both delay development and be lethal, because more fail to emerge when later put at lower temperatures, at 85% RH than at 95% RH. None emerged at 75% RH (see Table VII).

The ability of the midges to breed varied with the temperature. Those emerging at 10° were of no value as breeding stock. Those at 15° were quite suitable, so were those emerging at 20°. But increasing numbers of those emerging after being at 25°, 30° and 35° were malformed.

An apparently uniform stock of puparia can only be obtained during the autumn in temperate climates. In hotter regions where active breeding slows down in the summer as well as in the winter it may be possible to produce such stocks twice a year.

The effect of storage at 0° and 5° is not yet known.

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ZUSAMMENFASSUNG

ÜBER FAKTOREN, WELCHE DAS SCHLÜPFEN ÜBERWINTERNDER HESSENFLIEGEN-LARVEN BEEINFLUSSEN

Eine einfache Methode zur Erlangung von großen Mengen überwinternder Larven der Hessenfliege und Methoden für die Untersuchung des Einflusses konstanter Temperaturen und relativer Feuchtigkeit auf dieselben werden beschrieben.

Ein hoher Schlupf-Prozentsatz wurde bei einer Temperatur von 15° C und bei einer relativen Feuchtigkeit von 75, 85 und 95% erhalten, gleichgültig ob das Material dem Frost ausgesetzt war oder nicht. Bei Temperaturen von 20 und 25° wurde ein höherer Prozentsatz von älterem gefrorenen Material erhalten. Bei 30° schlüpften jüngere, nicht gefrorene Puparien (Scheinpuppen) nicht, obwohl ältere Puppen von gefrorenem Material zum Schlüpfen stimuliert wurden. In beiden Fällen erfolgte das Schlüpfen der Mücken nach Übertragung in eine Temperatur von 20°. Kein Schlüpfen erfolgte bei 35°, doch wurden dabei nicht alle Insekten getötet, da ein gewisses Schlüpfen zustande kam, wenn die Puparien wieder niedrigen Temperaturen ausgesetzt wurden.

Bei 20° und 95% relativer Feuchtigkeit nahm die Reaktionsgeschwindigkeit mit der Annäherung des Frühlings zu. Unter diesen Verhältnissen begannen die Puparien Anfang November nach 12—13 Tagen zu schlüpfen, während Anfang April das Schlüpfen schon nach 3—4 Tagen begann; ein 50%-Schlupf wurde nach 19 Tagen bzw. nach 7 Tagen beobachtet.

Es wurde kein Beweis dafür gefunden, daß Frost die Entwicklung beschleunige. Bei 24 Stunden Licht wurde das Schlüpfen schneller beendet als bei 8 und 16 Stunden oder ohne Licht.

Im Frühjahr erfolgte schwacher Schlupf bei 10° und keiner bei 5° und 0°.

Das Bespritzen der Puparien mit „Dowicide" verhinderte das Schlüpfen nicht.

Angaben über die Ausbildung der Puparien und das Schlüpfen im Frühjahr von ähnlichem Material, das in offenen Glashäusern überwintert wurde, sind zu Vergleichszwecken angeführt.

Es kann gefolgert werden, daß sich die vollernährten Larven in den Puparien während des Winters langsam entwickeln und auf experimentelle Umstände von konstanter Temperatur und Feuchtigkeit umso schneller reagieren, als der normale Zeitpunkt der Bildung von Puparien bzw. das Schlüpfen herannaht.

Für die Erzeugung von Mücken zu Versuchszwecken werden eine konstante Temperatur von 15° oder 20° C und eine relative Feuchtigkeit von 95% empfohlen.

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OBSERVATIONS ON ADULT WEIGHT AND WING AREA IN *PLUSIA GAMMA* L. AND *PIERIS BRASSICAE* L. IN RELATION TO LARVAL POPULATION DENSITY

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Crowding of lepidopterous larvae can influence adult morphology and physiology. With *P. gamma* and *P. brassicae* increasing emergence weight was not accompanied by proportionately as great increases in wing area. Larval crowding decreases both weight and wing area and accentuates the disproportion in the relation between wing area and weight. In the two species fore- and hind-wings are differently affected and the sexes were also affected differently. A warmer season also decreases fore- and hind-wing areas but distinct differences exist in the relative extent of the decreases. To obtain adequate data, both fore- and hind-wings must be measured.

Insect migration is a frequent sequel to crowding in immature stages. Although crowding may not induce the urge to migrate, it possibly contributes towards migration by its effect on form and function. In Lepidoptera, crowding reduces the weight of the larvae of *P. gamma* (LONG, 1953) and the weight of the resulting adult in most of the species that have been studied (HIRATA, 1956; IWAO, 1956; LONG & ZAHER, 1958). LONG & ZAHER (1958) found in *P. gamma* and *P. brassicae* that the adults had small bodies and small wings. Small size, however, does not necessarily imply a weaker flier. *P. gamma* frequently arrives in the British Isles accompanied by the Rush Veneer (*Nomophila noctuella* Schiff.) which is less than half the size of *P. gamma* (WILLIAMS, 1958).

The relation between wing area and weight in flying creatures has often been studied (LENDENFIELD, 1904; HOFF, 1919; PORTIER & DE RORTHAYS, 1926; PIERON, 1927; BANKS, 1930; OSBORNE, 1951) and various attempts have been made to express it mathematically. The wing area does not increase in proportion to increase in weight, so that the larger the species of animal the greater is the weight per unit area of wing surface. The ratio of total weight to wing area has often been termed "wing loading". In the consideration of the effects of larval crowding, it is important to know, therefore, whether wing loading increases with increasing emergence weight within a species and whether changes in weight induced by crowding are accompanied by equivalent changes in wing loading. In this paper these problems are considered and also whether adequate data could be obtained by the former technique used by LONG & ZAHER (1958) of measuring certain specified dimensions of the body and fore-wings.

TECHNIQUE

Two generations each of *P. gamma* and *P. brassicae* were bred in an unheated insectary as previously described for the work on similar cultures (LONG & ZAHER, 1958). The first generation (I) was in June/July 1956, the mean minimum temperature being 12.5° C and the second generation (II) in August/September with a mean minimum temperature of 8.2° C.

After voiding the meconium and within 12 hours of emergence perfectly formed adults of both species in generations I and II were killed in ether vapour and immediately weighed. The wings were then removed and held flat between two microscope slides sealed together with Cellotape. The wing areas in *P. gamma* were determined by direct measurement under low power binocular using needle dividers and a Leitz graduated scale reading to 0.1 mm. The larger wings of *P. brassicae* could be measured quicker by weighing pieces of tracing paper cut to the appropriate shape.

In expressing the results the term 'wing loading' is used simply to denote the ratio between the total weight and the wing area and is not intended to have a direct aerodynamical implication.

TABLE I.

Mean values and standard errors for weight at emergence, wing areas and "wing loading" in newly emerged adults from solitary and crowded cultures.

	Generation No.	♂ Solitary	♂ Crowded	♀ Solitary	♀ Crowded
P. gamma					
Live weight	I	123.7 ± 4.9	113.0 ± 4.3	134.6 ± 4.0	115.6 ± 2.7
in mg.	II	144.5 ± 2.9	123.5 ± 2.2	140.5 ± 3.1	123.5 ± 2.3
Fore-wing-Area	I	199.8 ± 2.8	191.4 ± 3.5	194.2 ± 1.1	183.8 ± 4.4
in sq. mm.	II	211.8 ± 2.7	206.6 ± 1.6	210.4 ± 1.2	193.4 ± 2.2
Hind-wing Area	I	167.4 ± 3.4	164.3 ± 3.6	165.7 ± 1.9	156.4 ± 3.9
in sq. mm.	II	189.8 ± 2.4	189.7 ± 1.9	191.4 ± 1.8	180.8 ± 2.2
Total Wing	I	367.2 ± 5.3	355.7 ± 6.7	359.9 ± 2.5	340.2 ± 7.0
Area sq. mm.	II	401.6 ± 4.8	394.3 ± 2.9	401.8 ± 4.0	374.2 ± 3.6
'Wing loading'	I	33.6 ± 1.0	31.7 ± 0.8	37.0 ± 1.1	34.1 ± 1.1
mg./sq.cm.	II	36.0 ± 0.5	33.8 ± 0.5	34.9 ± 0.6	33.0 ± 0.6
Numbers of	I	18	11	22	11
individuals	II	31	30	34	24
P. brassicae					
Live weight	I	198.6 ± 6.5	155.7 ± 5.5	198.8 ± 14.9	157.2 ± 8.2
in mg.	II	214.0 ± 5.5	190.8 ± 4.2	216.9 ± 5.3	188.3 ± 2.3
Fore-wing Area	I	748.1 ± 13.7	691.4 ± 12.0	781.2 ± 26.7	710.9 ± 8.9
in sq. mm.	II	875.4 ± 11.1	741.6 ± 10.1	876.8 ± 11.4	803.5 ± 3.7
Hind-wing Area	I	813.4 ± 14.0	779.2 ± 13.8	863.5 ± 34.7	764.7 ± 12.4
in sq. mm.	II	965.2 ± 12.1	795.7 ± 12.2	993.7 ± 14.7	899.0 ± 18.5
Total Wing	I	1561 ± 27	1471 ± 21	1645 ± 61	1476 ± 21
Area sq.mm.	II	1841 ± 22	1537 ± 21	1871 ± 25	1703 ± 32
'Wing loading'	I	12.7 ± 0.3	10.8 ± 0.3	12.0 ± 0.9	10.6 ± 0.5
mg./sq.cm.	II	11.6 ± 0.2	12.4 ± 0.9	11.6 ± 0.7	11.0 ± 0.6
Numbers of	I	19	12	8	13
individuals	II	14	17	17	15

Results

Measurements

Table I shows measurements obtained with 181 adults of *P. gamma* and 115 *P. brassicae.*

The results confirmed that larval crowding (Long & Zaher 1958) was associated with a decrease in live weight and wing dimensions in terms of wing areas. In crowded cultures emergence weight was less variable in both species as shown by the smaller standard errors. There was a strong tendency in crowded cultures for the reduction of wing area not to be proportionately as great as that of weight. Thus, with one exception, the wing loading was smaller in crowded than in solitary cultures, these differences being frequently significant.

Emergence weight and "wing loading"

With harmonic growth, surface area would be expected to increase with the square of linear function, whereas volume or weight would increase as the cube.

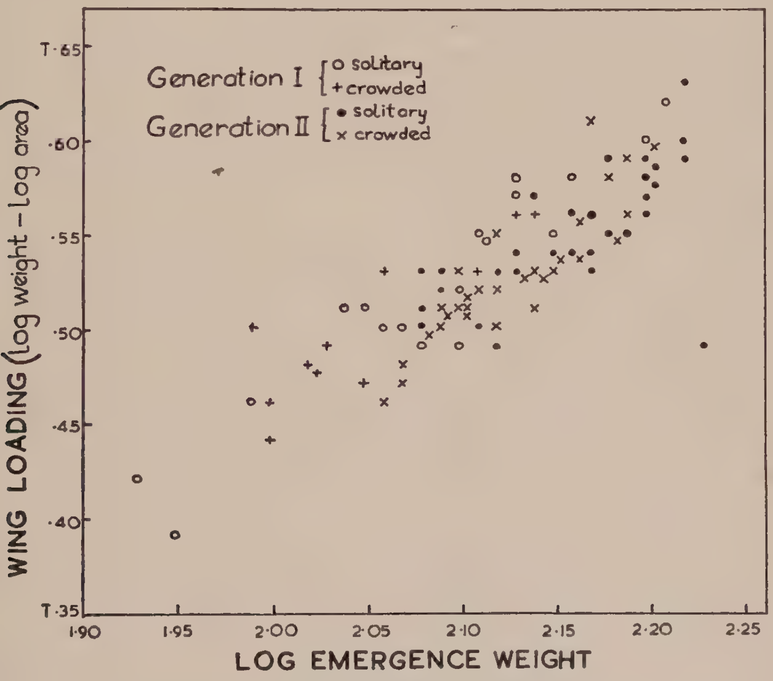

Fig. 1. The relation between wing loading and weight at emergence in two generations of *P. gamma.*

The wings of a moth may be regarded as 'extra surfaces', which would not come within this relationship, and analysis of the results shows that increases in weight are not associated with proportionately large increases in wing area. Hence wing loading increases with increasing emergence weight, as fig. 1 shows where wing loading expressed as log WEIGHT - log AREA is plotted against log EMERGENCE WEIGHT.

This relation held true for both sexes and both treatments (P = < 0.001). In both sexes the regression value for the crowded culture was greater than that for the solitary, whereas in both treatments that for the female was the greater. The regression values obtained for generation I were larger but were otherwise similar to those given for generation II in fig. 2.

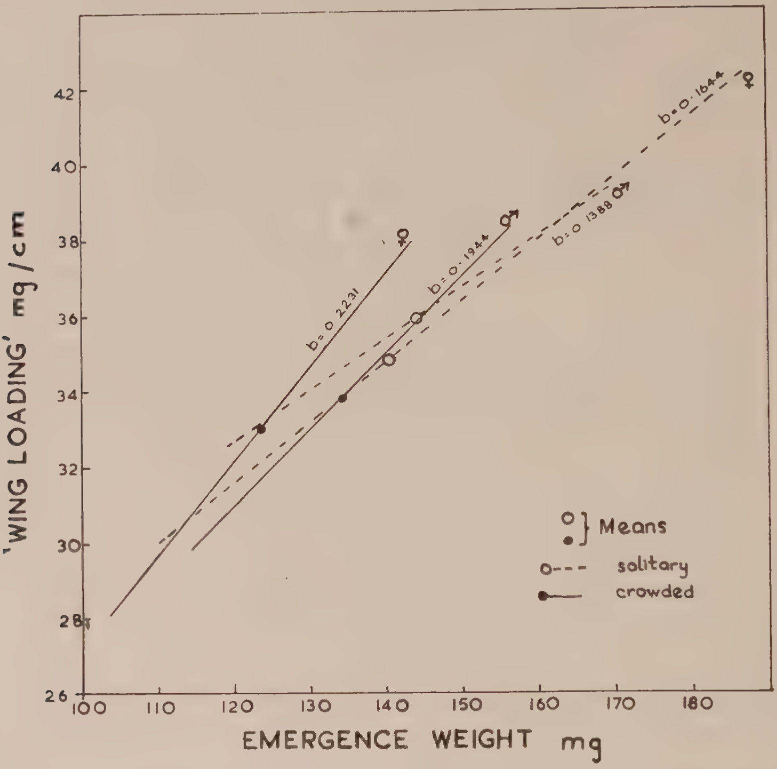

Fig. 2. 'Wing loading' regressions for adults from solitary and crowded cultures of *P. gamma* in relation to the weight at emergence. Total range of variation observed indicated by lengths of lines.

From this basic relationship between weight and wing loading it might be expected that adults from crowded cultures, which weigh less than their solitary counterparts, should therefore have the lower wing loading. With males three-quarters of the difference in wing loading could be attributable to the smaller weight, but the difference in wing loading actually observed in the females was little more than one half of that expected.

In *P. brassicae* a similar relation was observed between the weight at emergence and the wing loading, but the regression values were smaller, (Table II) and, unlike *P. gamma*, the values for the females were greater than those for the males only once.

TABLE II

Regression values (b) for the relationship between the weight at emergence and the 'wing loading' in P. brassicae

Sex	Generation	Solitaries	Crowds
♂	I	.03555	.05898
	II	.03668	.05842
♀	I	.03981	.04796
	II	.03410	.00313*

* Not significant at $P = < 0.05$

Observations on wing size

Comparisons of the areas of the fore- and hind-wings of *P. gamma* (Table I) showed the latter to be the smaller, ranging from 84 to 95 per cent of the former, and the crowded cultures showed the highest percentages, because crowding decreased the areas of the fore-wings more.

The wing reductions from crowding were significant in females but not in males (Table III).

TABLE III

Percentage reductions in wing areas produced by larval crowding.

		P. gamma		*P. brassicae*	
		♂	♀	♂	♀
Fore-wing	I	4.2	5.4	7.6*	9.0*
	II	2.5	8.1*	15.3*	8.4*
Hind-wing	I	1.8	5.6*	4.2	11.4*
	II	0.0	5.5*	17.6*	9.5*

* Significant at $P = < 0.05$

Generation I produced smaller adults than generation II, presumably because generation I was reared in warmer weather. In solitary cultures, where the effects of weather were not complicated by those of crowding, male weight was much affected whereas the fore- and hind-wing areas were most affected in the female. Consequently the wing-loading decreased in the males while it increased in the

TABLE IV

The percentage reduction of values obtained for generation II attributable to the warmer season and occurring in generation I

	Condition of culture	*P. gamma*		*P. brassicae*	
		♂	♀	♂	♀
Weight	Solitary	14.4	4.2	7.2	8.3
	Crowded	8.5	6.4	18.4	16.6
Fore-wing Area	Solitary	5.7	7.7	14.5	10.1
	Crowded	7.4	5.0	18.4	11.5
Hind-wing	Solitary	11.8	13.4	15.3	13.1
	Crowded	13.4	13.5	2.1	14.9
'Wing loading'	Solitary	6.7	–6.0	–9.4	–3.4
	Crowded	6.2	–3.3	12.1	3.6

females (Table IV). With the warmer season the decreases in the hind-wing areas of both sexes in *P. gamma* were greater than those produced by larval crowding.

Results with *P. brassicae* were quite different. In both sexes of this species the hind-wing was larger than the fore-wing, ranging from 107 to 113 per cent of the latter. The area of the hind-wing tended to be decreased more than the fore-wing by crowding, so that the higher percentages occurred in the solitary cultures.

Crowding did not appear to affect one sex consistently more than the other, but Table III suggests that the effects in the males may also be associated with temperature because their fore- and hind-wing areas decreased the least at the higher and the most at the lower temperatures. Table IV supports this, because, except for the male hind-wing area, other values were decreased most at the higher temperatures in the crowded cultures of males. The largest decreases in the crowded cultures of females occurred at the higher temperatures (Tables III & IV). Crowding accentuated the effect of the warmer season on weight by greatly increasing the percentage reduction in both sexes. Thus, in generation I, in both sexes there is a reduction in wing loading in the crowded cultures in contrast to an increase in the solitary cultures.

DISCUSSION

It is generally recognised that flight involves a delicate balance between several factors which include weight and wing dimensions. It is therefore unexpected that the relation between weight and wing area at emergence should vary considerably. In general the magnitude of growth responsible for the wing area is similar to that involving other body structures so that, for example, when the length of the femur is plotted against the square root of the wing area, a very definite relation can be obtained, although once again there is large individual variation. Thus in *P. gamma* and *P. brassicae* the effect is that wing loading increases with increasing weight. The higher regression coefficients obtained for wing loading on weight in crowded cultures indicates a distinct effect of crowding on the growth of the wings apart from its effect on body weight.

As in the previous study (LONG & ZAHER, 1958) differences were found in the effect of larval crowding between different species and between sexes of the same species. Thus crowding has the most effect on the area of the fore-wing in *P. gamma* and on the hind-wing in *P. brassicae* whereas in *P. gamma* the male hind-wing was little affected in contrast to a great effect on the female hind-wing. By confirming several of the effects observed in the earlier work, the results suggest that the former technique of measuring selected dimensions can give adequate data provided that both fore- and hind-wings are always included. The reference of HIRATA (1956) and IWAO (1956) to the effect of larval crowding on the ratio between body weight and fore-wing length as the effect on the "carrying weight" is therefore misleading. No effect on wing length could be established from their data.

In observations in *P. gamma* of weight changes occurring throughout adult life, to be published later, variations ranging from 49 to 164 per cent of the emergence weight were encountered. The wing loading at emergence obtained for *P. gamma* agrees well with the value of 33.0 mg/cm^2 cited by OSBORNE (1951). LENDENFELD (1904), HOFF (1919), PORTIER & DE RORTHAYS (1926), PIERON (1927), BANKS citing MULLERHOFF (1930) and OSBORNE (1951) give loadings for *P. brassicae* ranging from 8.0 to 12.1 mg/cm^2. These values are lower than those obtained in the experiments and therefore in view of known variations the importance must be stressed of noting other factors such as larval breeding conditions, season and adult age, in field data. Earlier workers tended to associate with the term 'wing loading' an aerodynamical implication based on the principle of a fixed wing in flight but later work has shown that other principles are involved in insect flight. Whereas not all of the wing surface need support the weight (WEIS-FOGH & JENSEN, 1956; WEIS-FOGH, 1956), the body surface has an aerofoil action (HOCKING, 1953). Considerable energy is expended overcoming wing inertia (SOTAVALTA, 1947) but this can be appreciably reduced by relatively small decreases in wing length (SOTAVALTA, 1952). At this stage, therefore, the results cannot be interpreted directly in terms of the effect of the morphometrical changes on flight performance.

I wish to thank Dr. K. Mellanby for reading this manuscript and I am grateful to Dr. Marjory G. Morris and Mr. M. J. R. Healy for their statistical advice and helpful discussions.

RÉSUMÉ

OBSERVATIONS SUR LE POIDS ET LA DIMENSION DES AILES CHEZ PLUSIA GAMMA *ET* PIERIS BRASSICAE *EN RELATION AVEC LE DENSITÉ DE POPULATION LARVAIRE*

Le groupement chez les larves de lépidoptères provoque la réduction du poids et des dimensions des ailes chez les adultes, et il est possible qu'elle contribue à provoquer la migration par son effet sur la morphologie et la fonction. Le rapport entre le poids total et la surface des ailes est souvent appelé "wing loading" et on sait que ce rapport croît avec l'accroissement de poids des différentes espèces animales qui volent.

L'effet du groupement larvaire sur le poids du corps et le "wing loading" chez *Plusia gamma* L. et *Pieris brassicae* L. a été étudié en comparant les adultes nouvellement éclos d'élevage de larves solitaires et de larves groupées. Le groupement réduit le poids et la surface des ailes d'une façon non proportionnelle, mais toutefois décroissante, au "wing loading". Pour chaque espèce et chaque sexe, le „wing loading" décroît pour un poids à l'éclosion décroissant, et ceci explique partiellement l'effet du groupement sur le "wing loading".

Chez *P. gamma* l'aile antérieure était plus grande que l'aile postérieure. Toutefois l'aile antérieure était la plus réduite en dimensions par le groupement, l'effet étant le plus marqué chez la femelle. La température affectait aussi le poids et la surface des ailes. Dans les élevages d'individus isolés, des températures plus élevées réduisaient principalement le poids des mâles, et la surface de leurs ailes postérieures, tandis que les surfaces des ailes antérieures et postérieures des femelles étaient plus affectées, de sorte que le "wing loading" décroissait chez les mâles et augmentait chez les femelles.

Chez *P. brassicae* l'aile postérieure est la plus grande et décroissait davantage avec le groupement. Le groupement ne paraissait pas affecter différemment les deux sexes, mais les résultats semblaient indiquer que l'effet pouvait être accentué chez les mâles et diminué chez les femelles quand les températures baissaient. Comme chez *P. gamma* des températures plus élevées diminuaient le poids et la surface des ailes chez *P. brassicae* mais du fait que

la surface des ailes était la plus affectée, le „wing loading" augmentait chez les deux sexes. Le groupement, cependant, accentuait l'effet de la température sur le poids tandis qu'elle réduisait le "wing loading". Ainsi chez les deux espèces, les réductions du poids et de la surface des ailes dûes à l'élévation de la température ne sont pas identiques avec les réductions dûes au groupement.

Les expériences ont montré également que, alors que les mesures directes de certaines dimensions pouvaient présenter une précision adéquate, les renseignements sur les ailes antérieures et postérieures devaient être groupés.

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STUDIES IN DIURNAL RHYTHMS IX
THE WATER-RELATIONS OF SOME NOCTURNAL TROPICAL ARTHROPODS

BY

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The rate of water-loss from tropical African centipedes (*Rhysida nuda* and *Ethmostigmus trigonopodus*) and millipedes (*Oxydesmus platycercus* and *Ophistreptus* sp.) is proportional to the saturation deficiency of the atmosphere. These animals therefore resemble forms from temperate and desert regions in this aspect of their physiology. Their nocturnal habits are probably related to their high rate of transpiration. The endogenous 24-hour rhythm shows a slight retardation in constant light and an acceleration in darkness. The probable function of this is to synchronize it with diurnal environmental changes. The West African scorpion, *Pandinus imperator,* has a 'critical temperature' considerably lower than those of desert species and comparable with that of the Italian *Euscorpius germanus.*

In recent years, the water-relations of terrestrial arthropods have aroused considerable interest (Edney, 1957), but surprisingly little information is available about water-loss by transpiration through the cuticle in tropical forms other than insects. The present paper presents data for centipedes, millipedes and scorpions from tropical Africa, and comparisons are made with information already available regarding species from desert and temperate regions.

MATERIALS AND METHOD

Experiments have been carried out on a number of scorpions, *Pandinus imperator* (C. L. Koch), scolopendromorph centipedes, *Rhysida nuda togoensis* Kräp. and millipedes, *Oxydesmus platycercus platycercus* Attems and *Ophistrepus* sp. kindly sent to me by air from Accra, Ghana in May, 1959 by my friend and former colleague, Dr. Freda Bowyer.

Unfortunately the scorpions were found to be in a moribund state on arrival but were kept in the refrigerator until required for experiment. In addition, water-loss data have been obtained from a large black centipede, *Ethmostigmus trigonopodus* (Leach), sent to me by air from Nyasaland in April 1955, by Dr. G. Fryer.

The rate of water-loss from different individuals in dry air at various temperatures was determined by weighing animals suspended over phosphorus pentoxide in conical flasks immersed in a water-bath. Specimens were exposed for 20 minutes at each temperature before the amount of water lost by evaporation was estimated.

Especially in species possessing a relatively impervious integument, there tends to be a conflict between the incompatible requirements of respiratory exchange and the prevention of water-loss (Cloudsley-Thompson, 1958).

Respiratory apertures are opened only when carbon dioxide accumulates within the body, so that transpiration is kept to a minimum. It has already been shown that water-loss is negligible through the lung-books of scorpions (CLOUDSLEY-THOMPSON, 1956) and it was felt that since the rate of transpiration through the cuticle of centipedes and millipedes is, in any case, comparatively high, to paint over the numerous spiracles of these animals might produce results more misleading than any obtained from intact specimens. The respiratory apertures of the creatures used in this investigation were therefore not blocked, as it was hoped to obtain figures that would shed light upon the ecological requirements of the living animals.

Results are expressed as mg./cm^2/hr., the surface area of the specimens being calculated from the formula $S = k\ W^{\frac{2}{3}}$ where S = surface area, W = weight and k is a constant. The value of k need only be approximate because, where specific differences in evaporation are great, an error of 50 per cent in its value will not affect the conclusions drawn (WIGGLESWORTH, 1945). A value of $k = 12$ was adopted for the centipedes in order that comparisons might be made with the results obtained by MEAD-BRIGGS (1956) who also used this value for *Lithobius forficatus* (L.) in England. In the case of the millipedes, a value of $k = 15$ was chosen so that results might be directly comparable with those obtained in England for *Oxidus gracilis* (C. L. Koch) by CLOUDSLEY-THOMPSON (1950), whilst for scorpions the value $k = 12$ allowed results to be compared with those previously obtained for species from Italy and the Tunisian desert (CLOUDSLEY-THOMPSON, 1956).

The experimental method is a crude one and has been criticised by EDNEY (1957) on the ground that no allowance is made for saturation deficit or diffusion effects. BEAMENT (1958) has also criticised it on five fundamental points, and particularly in that the temperature of the cuticle is substantially different from that of the surrounding air. Nevertheless, he has shown unequivocally that the 'critical temperature' of an epicuticular wax-layer is indeed a reality, the discontinuity observed corresponding with molecular disorganisation. Thus, although the absolute values obtained by the present experiments are certainly not reliable, this does not preclude valid conclusions being drawn when one species is compared with another. Furthermore, the adoption of a standardised technique has the merit of enabling comparisons to be made with previously published work of this kind.

Rhythms of locomotory activity have been determined by means of aktograph apparatus previously described (CLOUDSLEY-THOMPSON, 1956, 1958).

RESULTS

1. *Chilopoda*

It has long been known from field observations that centipedes are strictly nocturnal animals, but experimental confirmation has been obtained only in the case of *Scolopendra clavipes* C.L.K. which shows an endogenous rhythm of activity persisting for several days in darkness at constant temperature and humidity (CLOUDSLEY-THOMPSON 1956). Results obtained with *Rhysida nuda* are presented in Fig. 1. They prove that the rhythm persists under constant conditions, showing perhaps a slight retardation in light as is characteristic of the periodicity of nocturnal animals (ASCHOFF, 1958).

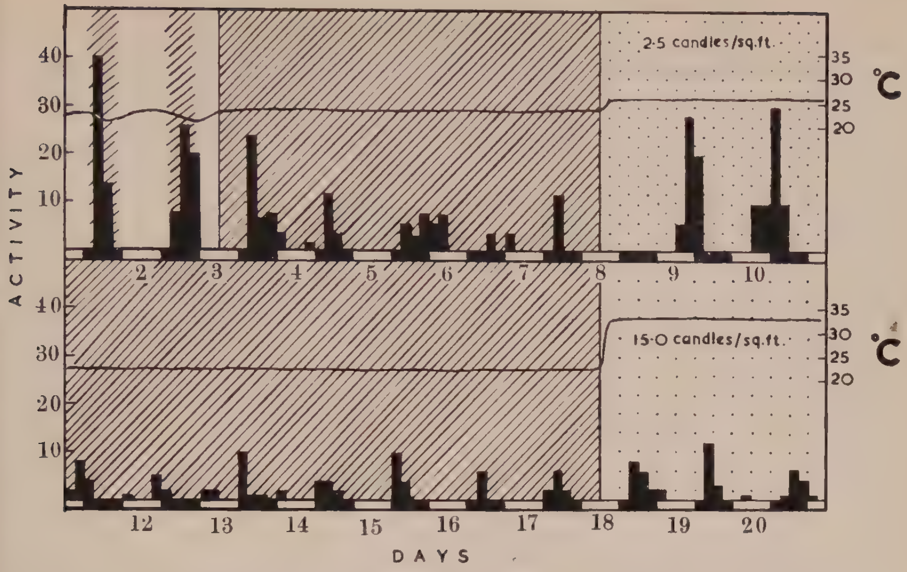

Fig. 1. Diurnal rhythm of locomotory activity in *Rhysida nuda* in a damp arena. Ordinates: activity on the left, temperature on the right. Abscissa: time in days. The white strips represent the 12-hour period from 06.00—18.00 hours. At first in normal daylight and darkness then in constant darkness and constant light (2.5 or 15.0 candles/sq.ft.).

The rates of water-loss in dry air from two specimens of *R. nuda,* weighing initially 0.696 and 0.263 gm respectively, are shown in Fig. 2. The lower abcissa indicates saturation deficiency in millimetres of mercury, the upper the corresponding temperatures. The higher figures were obtained consistently from the smaller specimen. Also included are data from *Ethmostigmus trigonopodus* (weighing initially 5.213 gm), *Lithobius forficatus* (data from MEAD-BRIGGS, 1956; not corrected for diffusion) and *S. clavipes* (mean of two specimens, weighing initially 1.538 and 0.960 gm — data calculated from CLOUDSLEY-THOMPSON, 1956). The smaller specimen of *S. clavipes* also showed a consistently higher rate of water-loss than the larger.

From this it is clear that a 'critical temperature' does not occur in any of the forms investigated, the rate of water-loss by transpiration through the cuticle and spiracles being always approximately proportional to the saturation deficiency of the atmosphere. It is noticeable, however, that the rate of water-loss from the desert species, *S. clavipes,* tends to be lower than that from tropical and temperate forms.

2. *Diplopoda*

It has already been demonstrated (CLOUDSLEY-THOMPSON, 1951) that the millipedes *Ophistreptus* sp. and *Oxydesmus platycercus* are nocturnal with an endogenous 24-hour rhythm. Recently, ASCHOFF (1958) has pointed out that my published figures indicate that the period of the rhythm of *Ophistreptus* is 24.8

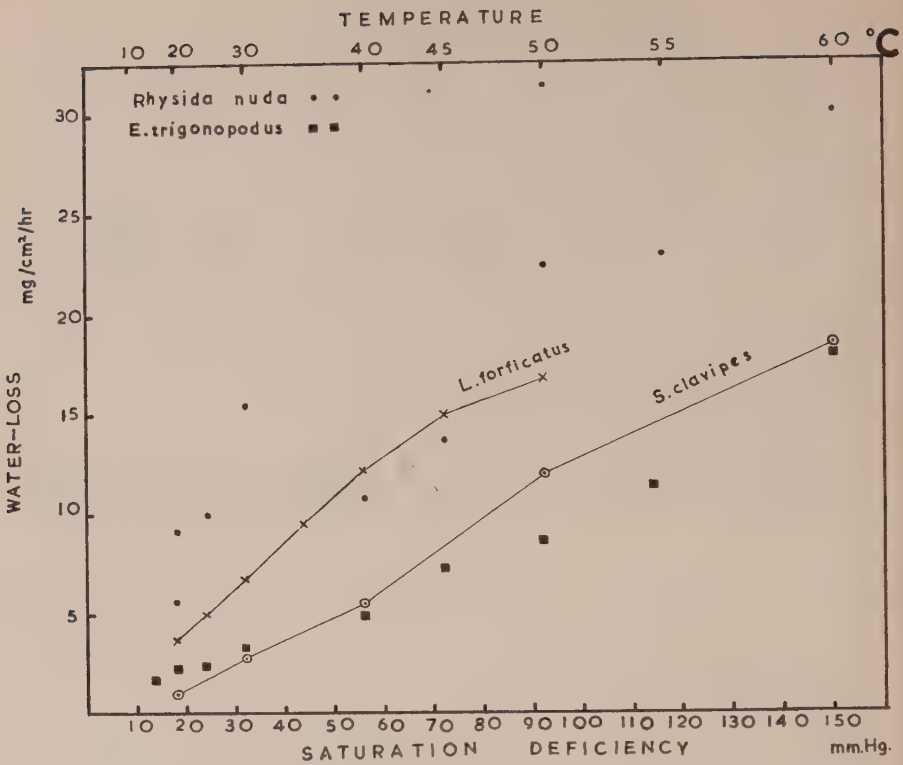

Fig. 2. Rates of water-loss in dry air at various saturation deficiencies and corresponding temperatures from *Rhysida nuda* and *Ethmostigmus trigonopodus* compared with *Scolopendra clavipes* and *Lithobius forficatus.*

hours in light and 23.0 hours in darkness. In other words, there is a slight retardation in light and an acceleration in darkness (cf. ASCHOFF, 1958).

Fig. 3 shows the rates of water-loss, in dry air at various temperatures, of *Oxidus gracilis,* a tropical species widely distributed in hot-houses throughout temperature regions (data from CLOUDSLEY-THOMPSON, 1950), the British *Tachypodoiulus niger* (Leach) (mean of 5 specimens, average initial weight 0.240 gm), *O. platycercus* (mean of 2 specimens weighing initially 0.757 and 1.175 gm) and *Ophistreptus* sp. (weighing initially 24.831 gm). Two exceptionally high readings obtained from this specimen, 25.8 and 50.9 mg/cm²/hr at 40° C and 50° C respectively, coinciding with discharge of fluid from the repugnatorial glands, have not been included in the figure.

No evidence of a 'critical temperature' can be seen in the water-loss curves of any of these species, transpiration being approximately proportional to saturation deficiency in each case.

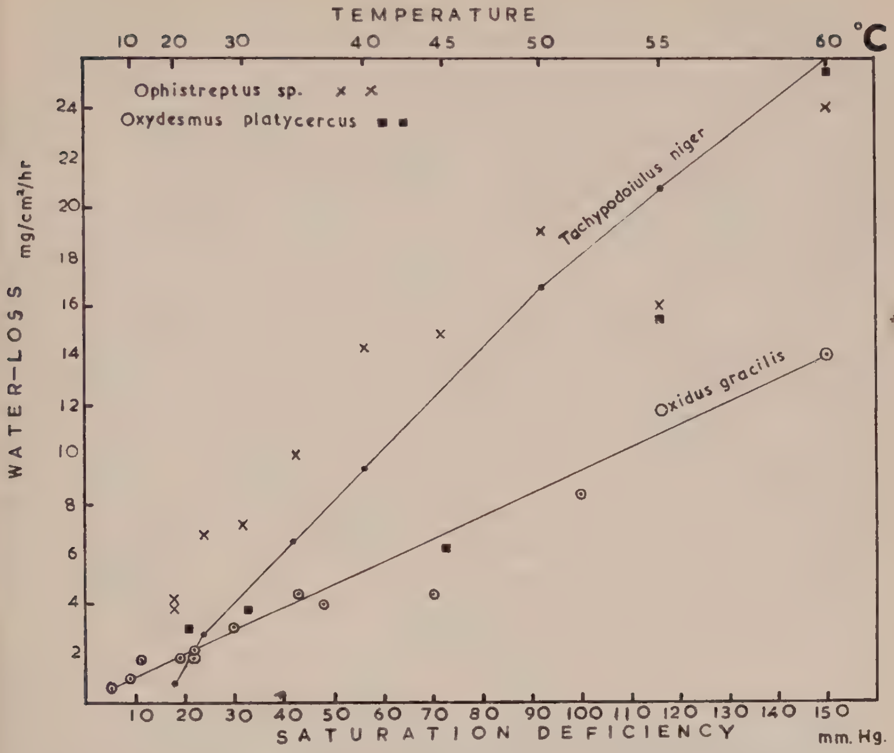

Fig. 3. Rates of water-loss in dry air at various saturation deficiencies from *Ophistreptus* sp. and *Oxydesmus platycercus* and corresponding temperatures compared with *Oxidus gracilis* and *Tachypodoiulus niger.*

3. *Scorpiones*

Fig. 4 shows rates of water-loss at different temperatures in dry air from five immature specimens of *Pandinus imperator* weighing initially 20.84, 18.09, 16.87, 15.30 and 14.89 gm (mean 17.20 gm). On the same graph are shown the rates of water-loss under similar conditions from the fat-tailed desert scorpion, *Androctonus australis* (L.) (mean weight 7.092 gm) and the Italian *Euscorpius germanus* Schaef. (mean weight 0.406 gm) (data from CLOUDSLEY-THOMPSON, 1956). From this it can be seen that the desert species has far greater powers of water conservation than the scorpions from tropical West Africa. Its 'critical temperature' is about 70° C whilst that of *P. imperator* is only about 50° C, the curve for *P. imperator* being almost identical with that of *E. germanus.*

A dead specimen of *P. imperator* weighing initially 19.998 gm showed an average loss in weight of 1.25 mg/cm2/hr. in dry air at room temperature (19° ± 2° C) over a period of five days. Under similar conditions a living specimen of *A. australis* averaged only 0.014 mg/cm2/24 hrs. (CLOUDSLEY-THOMPSON, 1956).

It may be objected that scorpions belonging to different families are being compared: *Pandinus* (family Scorpionidae), *Androctonus* (family Buthidae) and

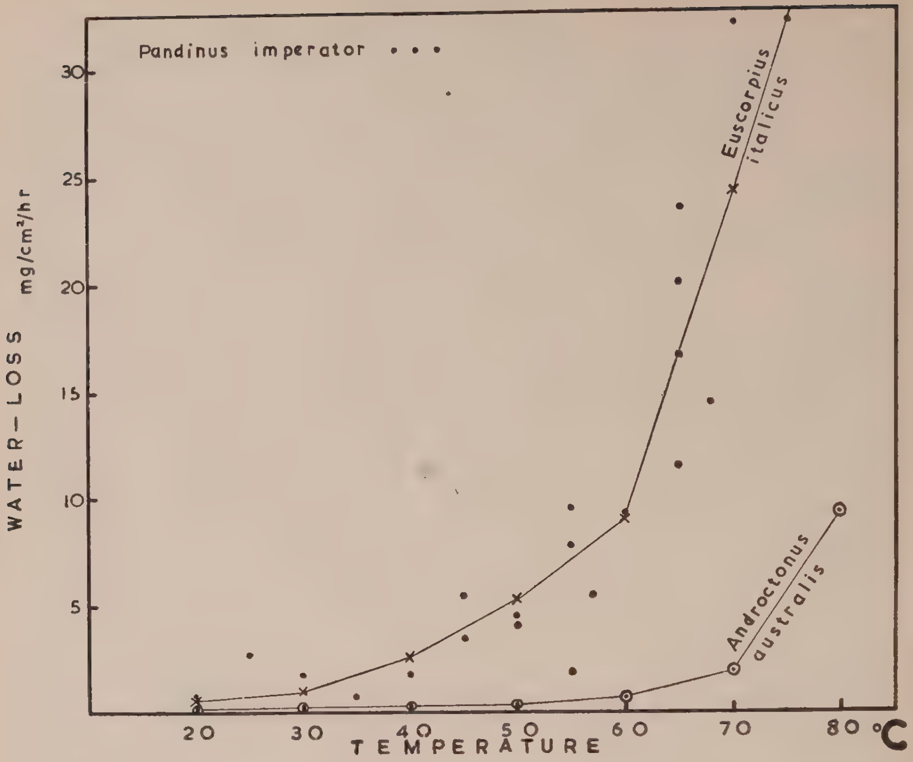

Fig. 4. Rates of water-loss in dry air at different temperatures from *Pandinus imperator* compared with *Androctonus australis* and *Euscorpius germanus.*

Euscorpius (family Chactidae); and that the differences observed might be related to systematic position rather than to habitat. However, *Scorpio maurus* L. (family Scorpionidae), a typical desert species, has a water-loss curve showing a 'critical temperature' about 65° C. (CLOUDSLEY-THOMPSON, 1956) and is comparable in this respect with *Androctonus* rather than the more closely related *Pandinus.* It seems probable, therefore, that the rates of water-loss in the different species are a genuine reflection of adaptation to habitat.

DISCUSSION

All the species studied in the present paper are markedly nocturnal in their habits. This is to be expected in the case of centipedes and millipedes from which water-loss is proportional to the saturation deficiency of the atmosphere, but it is more surprising in scorpions. The markedly nocturnal habits of most species of scorpions are therefore most probably the result of other ecological factors (in particular, vulnerability to vertebrate enemies) on account of their large size and slow movements, for they appear to be able to withstand high temperatures and low humidity for quite long periods.

However, the South African scorpion, *Opisthophthalmus latimanus* Koch, often comes to the mouth of its burrow during the daytime. This is one of the species in which characteristic 'stilting' or the elevation of the abdomen occurs as an adaption to temperature stress (ALEXANDER & EWER, 1958), which lends support to the hypothesis suggested above; for a purely nocturnal species would not require this behaviour pattern. Of course, it might be argued that scorpions are protected by their poisonous stings. Poison is not always an effective deterrent to large and powerful enemies, however, which might well trample a scorpion underfoot, just as deer will stamp on a snake. Again, baboons and other monkeys often become adept at catching scorpions without themselves getting stung. Elsewhere I have argued that the 'pectines' are sensory receptors for vibrations of the ground and serve more for the avoidance of enemies than for the detection of prey (CLOUDSLEY-THOMPSON, 1958). ALEXANDER (1958) records a number of mammalian predators of *O. latimanus* including rats, shrews, mongooses and hedgehogs under laboratory conditions.

Comparison of the rates of water-loss from tropical centipedes, millipedes and scorpions with rates from species inhabiting temperate and desert regions suggests that transpiration is, to some extent, related to habitat, being considerably lower in desert species.

My thanks are due to Dr. F. A. Turk for identifying the 'myriapod' material.

ZUSAMMENFASSUNG

UNTERSUCHUNGEN ÜBER DIURNALE RHYTHMEN IX. DIE WASSER-VERHÄLTNISSE EINIGER NÄCHTLICHER TROPISCHER ARTHROPODEN

Bei den tropischen afrikanischen Hundertfüßlern *Rhysida nuda* und *Ethmostigmus trigonopodus*, den Tausendfüßlern *Oxydesmus platycercus* und *Ophistreptus* sp. sowie bei dem Skorpion *Pandinus imperator* wurden die Wasserverlustraten durch Wägen der Individuen bestimmt, die über Phosphorpentoxyd in konischen Flaschen aufgehängt wurden, die in ein Wasserbad tauchten. Die Tiere wurden jeder Temperatur 20 Minuten lang ausgesetzt, bevor der durch Evaporation bedingte Betrag des Wasserverlustes bestimmt wurde. Es wird gezeigt, daß diese Methode wertvolle Vergleiche zwischen den verschiedenen Arten zu ziehen gestattet. Sowohl bei Hundertfüßlern wie auch bei Tausendfüßlern erwies sich die Transpiration als proportional zum Sättigungsdefizit der Luft. Diese Tiere ähneln deshalb in diesem Punkte ihrer Physiologie Arten aus gemäßigten und Wüsten-Gebieten.

Ihre nächtliche Lebensweise, die mittels Aktographen festgestellt wurde, steht wahrscheinlich in Beziehung zu der vergleichsweise hohen Wasserverlustrate. Der endogene diurnale Rhythmus zeigt eine leichte Verzögerung bei Dauerbeleuchtung und eine Beschleunigung bei Dunkelheit. Auf diese Weise wird wahrscheinlich die Periodizität dieser Tiere mit dem 24-Stunden-Rhythmus der Umgebung synchronisiert.

Die kritische Temperatur des westafrikanischen Skorpions *Pandinus imperator* liegt beträchtlich niedriger als die der Wüsten-Arten und ähnelt der des italienischen *Euscorpius germanus*. Es wird vermutet, daß in diesem Falle die nächtliche Lebensweise in Beziehung steht zu der Gefährdung des großen, langsamen Tieres durch räuberische Wirbeltiere.

Es wird geschlossen, daß im allgemeinen die Transpirationsrate in Beziehung zum Habitat steht und bei Wüstenbewohnern am niedrigsten ist.

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THE ECOLOGY OF SYMPHYLA. PART II. SEASONAL SOIL MIGRATIONS

BY

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The seasonal changes in vertical distribution of *Scutigerella immaculata* Newport in the soil were studied. In bare soil outdoors there were high surface numbers in spring, low in summer, with a second increase in autumn. The distribution pattern was similar in bare greenhouse soil, except that spring migrations to the surface soil occurred earlier, the summer exodus was more marked, and the autumn increase much reduced. Growing plants attracted *Scutigerella immaculata* to the surface even under adverse soil conditions but had no influence on *Symphylella vulgaris* Silvestri. The results are discussed in relation to other work.

In an earlier paper, evidence was given that symphylids are common soil animals in South West England (EDWARDS, 1958). They are found in a wide range of soil habitats and under suitable soil conditions very large populations may occur. It was indicated that symphylids differ from many other soil animals in that they commonly penetrate several feet into the soil, and their downward migrations were limited only by the presence of a clay subsoil, or some other barrier or soil condition.

In early soil faunal studies it was suggested that soil arthropods were mainly confined to the upper few inches of soil (THOMPSON, 1924), (FORD, 1935), and early workers on the Symphyla also made this assumption (NEWPORT, 1845), (GRASSI, 1886), (HANSEN, 1903), (FRIEDEL, 1928). Later work (DIEM, 1903), (EDWARDS, E. E. 1929), (SALT, HOLLICK, RAW & BRAIN, 1948) showed symphylids to be very common at much lower levels.

Seasonal vertical migrations were first reported by WYMORE (1924), and were shown to be common by RILEY (1929), WALTON (1930), KEARNS & WALTON (1932), MILES (1935) and MARTIN (1948). These were only general observations and the first numerical date in the literature is by SAWA (1930), in a study of the soil fauna of arable and pasture land in Japan, but unfortunately he did not distinguish between species of symphylids. He said that symphylids do not undergo any marked seasonal migrations in soil, but his results do not support this view and Figure 1, which was plotted from his data shows a marked decline in numbers of symphylids in the surface soil during the summer. This may be a temperature reaction since the summers in Japan are warm but with moderate rainfall.

MICHELBACHER (1939) made a thorough study of the seasonal changes in the vertical distribution of two species, *Scutigerella immaculata*, and *Symphylella*

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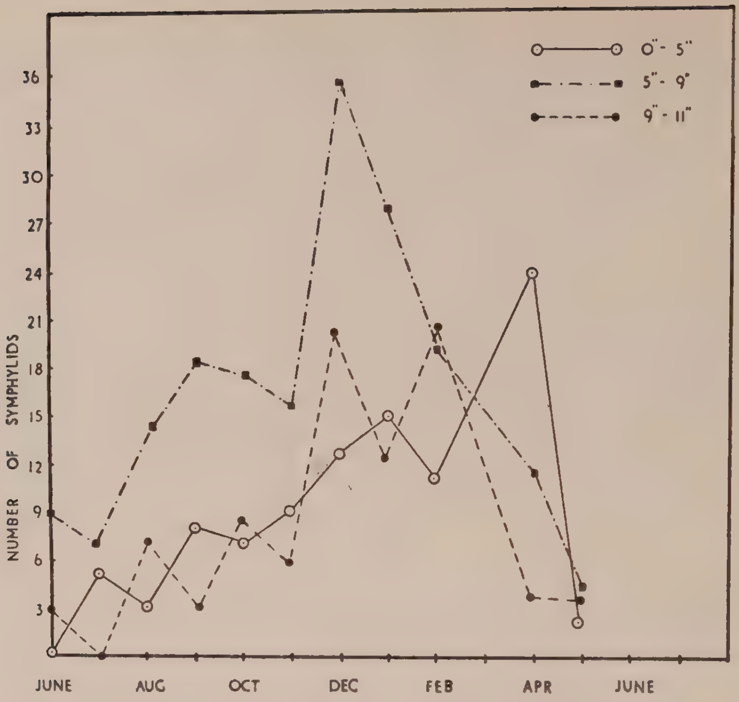

Fig. 1. Seasonal changes in distribution of Symphylids in Japanese arable land (From data by SAWA, 1930).

subterranea Michelbacher, in arable soil in California. He found that the former species was most numerous at the soil surface from February to May and July to September, moving to the lower layers during the hot summer months. *S. subterranea* was almost entirely confined to the lower layers of soil throughout the year and he related this to the saprophagous habits of this species. Recently, interesting data on changes in the vertical distribution of symphylids in West African pasture, were given in a paper by BELFIELD (1956). He recorded a gradual increase in monthly rainfall from 0.01 inches in December to 7.31 inches in May and there was a corresponding increase in numbers of symphylids in the surface 6 inches of soil (Figure 2).

The present study was undertaken because no information was available on the soil movements of symphylids under European climatic conditions. Two species of symphylids were studied, only one of which was of economic importance, and the differences between their soil migrations in greenhouse soil and in field soil were investigated. No attempt was made at this stage to analyse the individual soil factors responsible for the seasonal migrations.

SAMPLING SITES

Sites were chosen with large and comparable populations of two species of symphylids. The species were *Scutigerella immaculata* Newport, which is om-

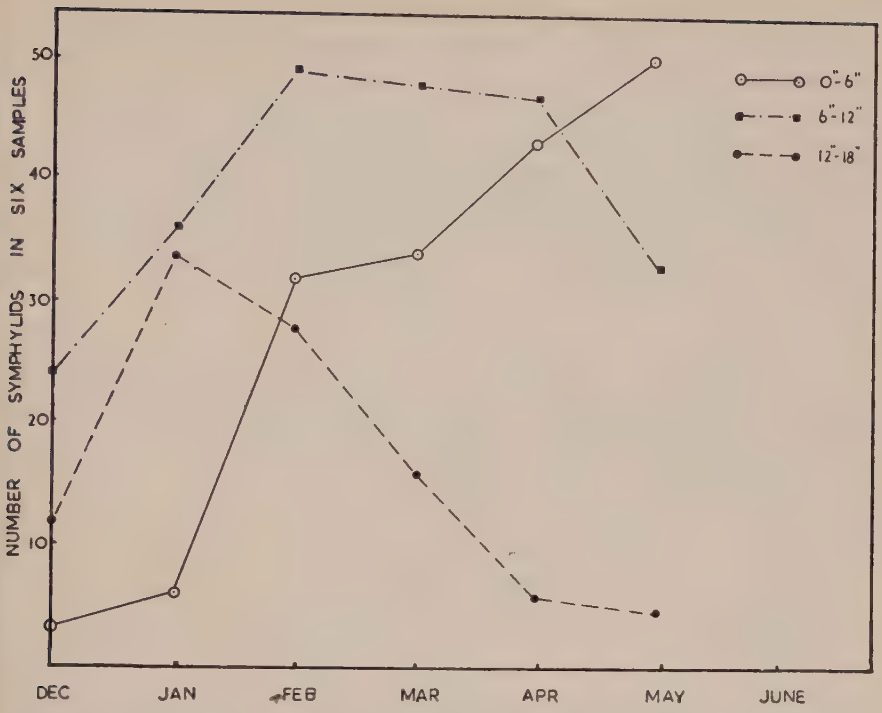

Fig. 2. Seasonal distribution of Symphylids in West African pasture (from data by Belfield, 1956).

nivorous and an economic pest due to its preference for plant food in the form of young growing roots, and *Symphylella vulgaris* Silvestri, which is believed to subsist on decaying matter and micro-organisms present in the soil.

1. *Open Soil*

A smallholding with a clay loam soil which had borne a strawberry/anemone rotation for a number of years. During the sampling period the land was cropped with second year strawberries, which fruited in June.

2. *Greenhouse Soil*

A greenhouse with sandy loam soil growing tomatoes and occasionally chrysanthemums. The house was planted with tomatoes from January to September of the sampling period and left uncropped until December. Consequently during the early part of this time artificial soil heating was in operation. After the tomato crop was finished the root systems were left in the soil to enable sampling to be completed.

SAMPLING TECHNIQUE

At each site 5—10 soil samples were taken at approximately monthly intervals for twelve months. Samples consisted of vertical cores taken with a 2½ inches deep, 2½ inches diameter, cylindrical sampling tool, which had a long handle to allow samples to be taken from the lower soil levels. Each complete core consisted of a series of successive 3 in. deep, cylinders of soil which were separately immersed in water and then stirred to free the symphylids. The animals floated to the surface of the water where they could be picked off with a fine brush and counted. As far as possible the samples were taken close to previous ones to obtain comparable data. Those samples which related to plants were taken as cores through the root systems and the others in open soil between the plants.

RESULTS

The data obtained from the sampling are summarized in Figures 3—8, the sampling data being split up into three soil levels of 0—6, 6—12 and 12—18 inches. In each graph the heavy continuous line shows the changes in numbers of symphylids in the upper 6 in. of soil.

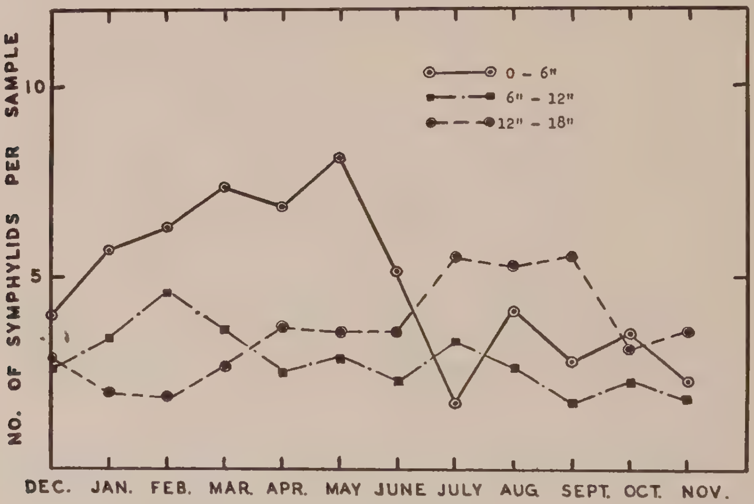

Fig. 3. Vertical seasonal distribution of *Scutigerella immaculata* in bare greenhouse soil.

(i) Scutigerella immaculata *in greenhouse soil*

In bare soil, (Figure 3), there was an increase in surface numbers of symphylids in spring to a peak population in May, followed by a very rapid decline to a minimum level in July, and numbers only increased very slightly again during autumn. It is possible that the low surface numbers recorded in July were due to the warm, dry soil conditions found at this time.

This pattern was very much more pronounced when samples were taken close to the root systems of a tomato crop (Figure 4). In this case there was an extremely rapid build up in surface numbers to as much as three times that in bare soil, within a week of the tomatoes being planted in late January. This can

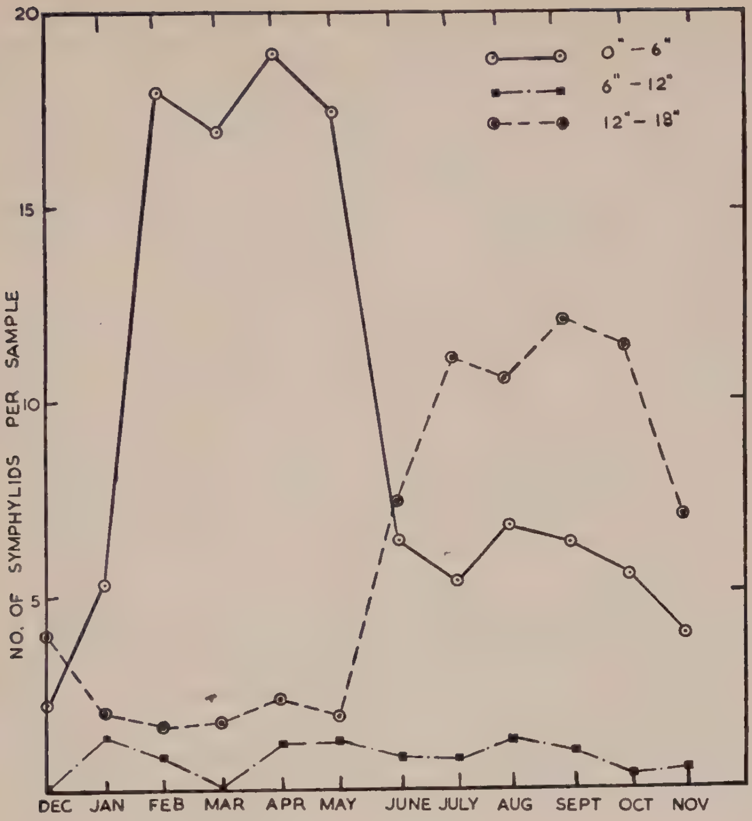

Fig. 4. Vertical seasonal distribution of *Scutigerella immaculata* under tomato plants in greenhouse soil.

only be accounted for by lateral migration from the bare surface soil to the tomato roots. The high surface population was maintained until May, when there was once again a very marked decrease in surface numbers similar to that found in the samples taken in bare soil. The period of high surface numbers coincided quite closely with that of high production of young growing roots by the tomato plants.

(ii) Scutigerella immaculata *in field soil*

In this case the movement of symphylids to the bare surface soil was much more gradual, (Figure 5), but high numbers were found at the surface in the period from February to June. The surface numbers were low in July and August, but markedly increased during early autumn. This migration back to the surface in autumn contrasted with only a slight rise in surface numbers seen in greenhouse soil in the corresponding period. The fall off in surface numbers in summer was later outdoors than in the greenhouse soil, and less pronounced, probably due to the earlier and more extreme physical changes which occur during this period in greenhouse soil.

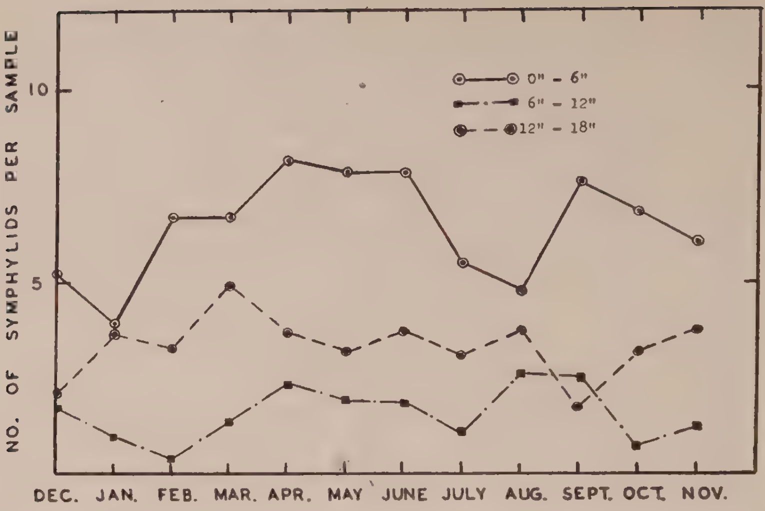

Fig. 5. Vertical seasonal distribution of *Scutigerella immaculata* in bare soil outdoors.

When samples taken close to the strawberry plants are considered (Figure 6) the distribution pattern is markedly different. The surface numbers did not increase until May, were maintained in June and July and decreased only slightly in August. High surface numbers were attained again in autumn. The highest surface population occurred much later outdoors, and the autumn population was concentrated much closer to the surface than in greenhouse soil.

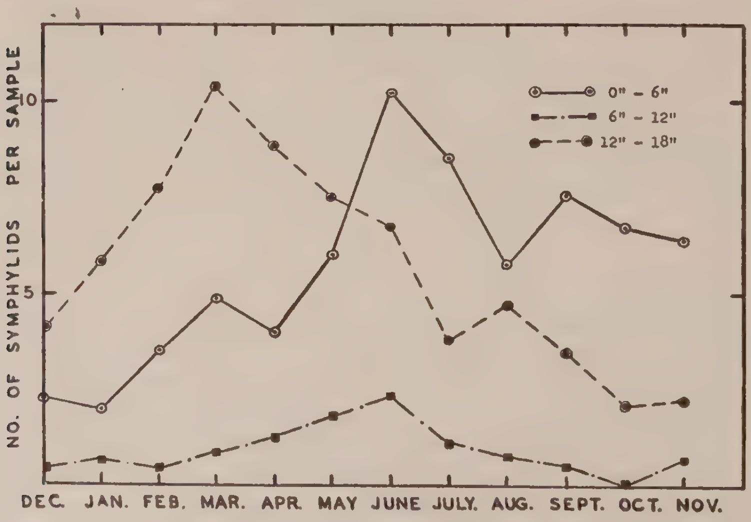

Fig. 6. Vertical seasonal distribution of *Scutigerella immaculata* under strawberry plants outdoors.

(iii) Symphylella vulgaris *in greenhouse soil*

The surface numbers of symphylids under tomato plants showed a gradual increase in spring and early summer, and a gradual decrease from May onwards

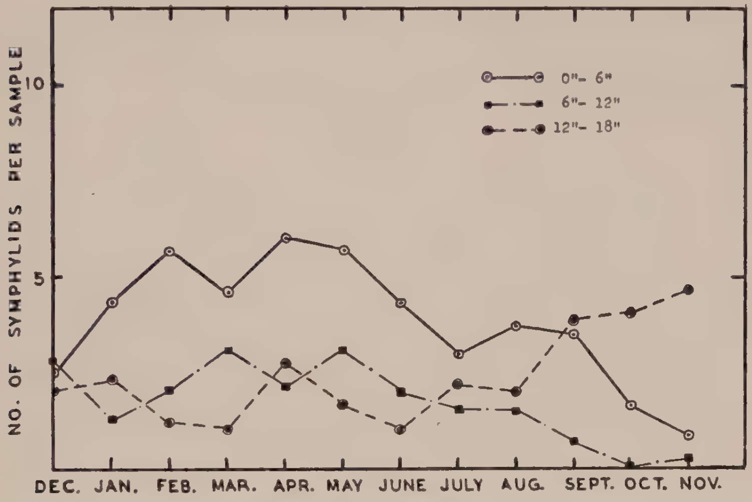

Fig. 7. Vertical seasonal distribution of *Symphylella vulgaris* under tomato plants in greenhouse soil.

(Figure 7). The pattern closely resembled that of *Scutigerella immaculata* in bare greenhouse soil, although the decrease in surface numbers which occurred between May to July was not so marked.

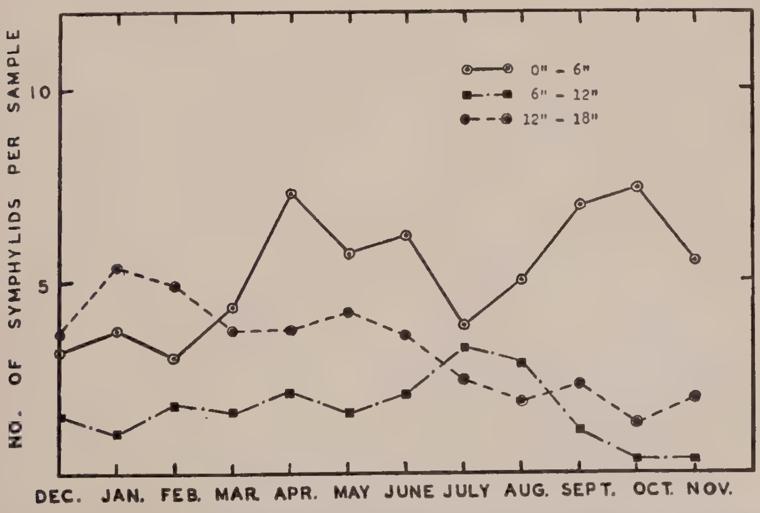

Fig. 8. Vertical seasonal distribution of *Symphylella vulgaris* outdoors under strawberry plants.

(iv) Symphylella vulgaris *in field soil.*

The surface population of this species under strawberry plants remained low until March, reached its highest level in April and lowest numbers were found in July (Figure 8). There was a secondary build up in surface numbers in autumn to another maximum in October followed by a further decrease. This pattern was rather similar to that of *Scutigerella immaculata* in bare field soil except that this species migrated to the surface somewhat earlier in spring.

DISCUSSION

The *total* numbers of *Scutigerella immaculata* in greenhouse and field soil at all depths, showed marked seasonal variations (Figure 9). Michelbacher (1939) found that symphylids breed all the year round, but the populations shown in Figure 9 indicate that in England, reproduction is at a maximum during the spring and summer. This was borne out by the greater percentage of young symphylids present in spring samples. Total numbers increased more rapidly in the spring in greenhouse soil, probably in response to the warm and moist soil, and availability of food. The fall off in numbers in greenhouse soil in June and

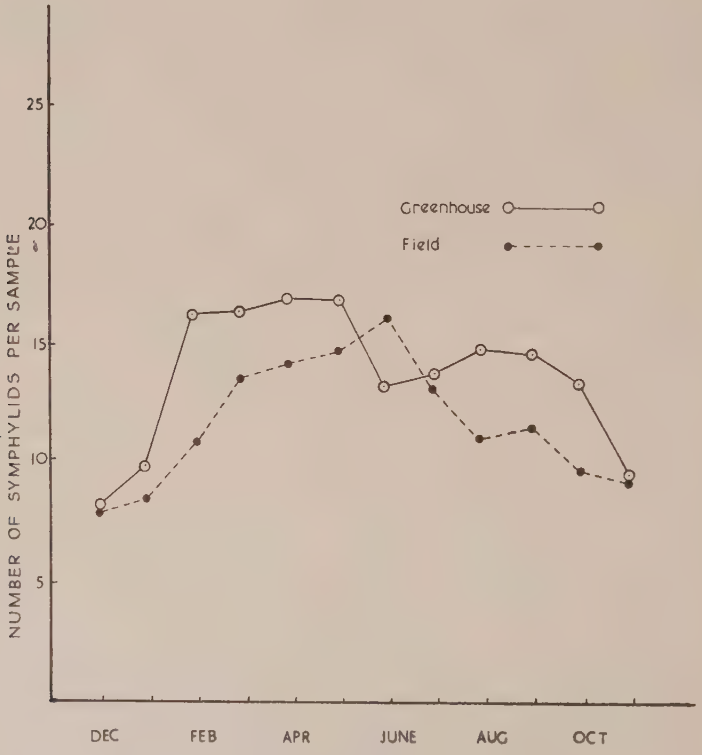

Fig. 9. Seasonal changes in total numbers of *Scutigerella immaculata* at two locations in S.W. England.

July could be due to the very warm surface soil which may have killed some symphylids or forced them down to lower soil levels with the result that they were deprived of food.

These seasonal changes in total numbers of symphylids present in the soil were accompanied by changes in the vertical distribution of the population. Figures 3—8 summarize the changes in the present work, and similarities and contrasts in in these graphs may indicate possible factors responsible for the migrations.

A characteristic of the vertical distribution of both species at all times in the year was the relatively small number of symphylids found in the six to twelve inch soil stratum. Probably symphylids found in this zone were migrating between the surface soil and the subsoil. A second feature common to all habitats was that the surface numbers of symphylids were at their lowest level in mid-winter, with a gradual increase in spring, and a decrease during the summer, sometimes followed by a second increase in autumn. It can be concluded that this pattern develops in response to the occurrence of optimal soil conditions at the surface at certain times in the year.

Some indication of the relative importance of factors which could influence the migrations of symphylids to the soil surface may be obtained by comparison of differences between certain of the seasonal vertical distribution graphs.

The graphs for *Scutigerella immaculata* in bare greenhouse soil (Figure 3) and in bare field soil (Figure 5) demonstrate the effects of different environmental conditions on the seasonal migrations of a single species. Under greenhouse conditions the surface numbers increased earlier in the year, the summer decrease was more marked, and there was little secondary increase in the autumn. This may have been due to the occurrence of optimal soil conditions earlier in the year in the greenhouse soil, while adverse conditions in summer and autumn resulted in low surface populations at these times.

Differences in seasonal vertical distribution between the two species under the same set of environmental conditions are demonstrated by comparison of Figures 6 and 8, in this case under strawberry plants outdoors. *Scutigerella immaculata* reached much higher surface numbers than *Symphylella vulgaris* during early summer, and these differences were maintained into the early autumn. A similar comparison can be made for the two species under tomato plants in the greenhouse (Figures 4 and 7). Here even more marked differences can be seen with extremely high numbers of *Scutigerella immaculata* present under the roots of tomato plants in early spring, in the same samples that yielded comparatively small numbers of *Symphylella vulgaris*. Probably these differences may be accounted for by the feeding habits of the two species, *S. immaculata* being strongly attracted to young roots, both laterally and vertically.

The effect of young roots in the surface soil on the numbers of *Scutigerella immaculata* is demonstrated by comparison of Figures 3 and 4. Very much higher numbers of symphylids were found at the surface in spring when young roots were abundant, than in the bare soil. Conversely the presence of young roots had little effect on the surface numbers of *Symphylella vulgaris* (Figures 7 and 8).

These observations all indicate that the seasonal vertical migrations of symphylids may be due to several factors. Phytophagous species such as *Scutigerella* *immaculata* probably respond to the presence of young roots even when other factors are adverse. This species in the absence of plants behaves similarly to *Symphylella vulgaris* and their migrations must be in response to changes in such factors as soil temperature and moisture. Certainly a hot dry soil appears to bring about migration of symphylids to more suitable conditions in the lower soil, and to a lesser extent a very cold soil has the same effect.

These results are in relatively good agreement with the sparse data available in the literature. MICHELBACHER (1939) found low numbers of *S. immaculata* in the surface soil from May to July, when the soil would be hot and dry in California. Similarly, in the data given by SAWA (1930) the lowest surface numbers were from May to August, the warm summer period, although in this case the soil would be relatively moist, so that temperature may be the more important factor controlling the migrations. In West African pasture, (BELFIELD, 1956) there was a gradual rise in surface numbers of symphylids from December to May, and in this period there was a change of only 5° F in the average monthly temperatures, but there was a gradient in average monthly rainfall from practically zero to approximately seven inches in the same period. Here moisture must have been the factor influencing migrations.

It can be concluded that symphylids prefer a warm moist soil with adequate food material, and under these conditions it is probable that large numbers will be found in the surface soil. Under other conditions the great majority of the total population will be found in the deeper layers of soil.

ZUSAMMENFASSUNG

DIE ÖKOLOGIE DER SYMPHYLIDEN. TEIL II. JAHRESZEITLICHE WANDERUNGEN IM BODEN

Symphyliden dringen sehr tief in den Boden ein. Es wurden die jahreszeitlichen Vertikalwanderungen von zwei Arten durch Entnahme einer Anzahl von Tiefenproben in monatlichen Abständen untersucht. Dies waren *Scutigerella immaculata*, die phytophag ist, und *Symphylella vulgaris*, die saprophag lebt. Die Fortpflanzung findet das ganze Jahr über statt, doch traten Maximalpopulationen im Gewächshausboden im Frühjahr und im Freiland im Frühsommer auf. Im Freiland wanderten auf offenen Böden große Zahlen beider Arten im Frühling und Frühsommer an die Bodenoberfläche, kehrten im Hochsommer in geringere Tiefen zurück, und im Herbst entstand ein zweiter kleinerer Gipfel der Oberflächenzahlen.

Ein ähnlicher jahreszeitlicher Verlauf zeigte sich in nacktem Gewächshausboden, außer daß die Frühjahrswanderung zur Bodenoberfläche zeitiger stattfand, der Sommerrückzug stärker betont war und im Herbst nur relativ wenige zur Bodenoberfläche zurückkehrten. Proben unter Pflanzen in Gewächshaus- und Feldböden zeigten größere Zahlen von *Scutigerella immaculata* zu den Zeiten, in denen die Pflanzen die größte Anzahl junger Wurzeln entwickelten. Das trat bei *Symphylella vulgaris* nicht auf. Es wird geschlossen, daß eine warme, feuchte Bodenoberfläche für hohe Oberflächenzahlen beider Arten am meisten geeignet ist, doch kommt *Scutigerella immaculata* sogar bei ungünstigen Bedingungen an die Oberfläche, wenn Nahrung vorhanden ist.

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ZUR LABORATORIUMSPRÜFUNG VON TEXTILIEN AUF TERMITENFESTIGKEIT MIT *KALOTERMES FLAVICOLLIS* FABR.

VON

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Für die Prüfung von Textilien auf Termitenfestigkeit hat sich seit langer Zeit die Trockenholztermite *Kalotermes flavicollis* Fabr. hervorragend bewährt. Durch neue radiobiologische und verhaltensphysiologische Untersuchungen wird die vorgeschlagene Prüfmethodik biologisch begründet und im einzelnen näher erläutert.

Biologische Verfahren zur Prüfung von Textilien sind überaus schwierig, da Art des Prüforganismus, dessen Herkunft, Vorbehandlung und physiologischer Zustand Einfluß nehmen können auf den Ablauf der Versuche. Dennoch zeigen gerade die mit Insekten durchgeführten Untersuchungen, daß bei vertiefter Kenntnis der Versuchstiere, insbesondere ihres Verhaltens und ihrer physiologischen Reaktionen, von Laboratorien mit genügend großen Spezialerfahrungen doch bis zu einem recht weitgehenden Grade reproduzierbare Prüfergebnisse erlangt werden können. Nach unserer Ansicht, die bereits mehrfach dargelegt wurde (GÖßWALD, 1941 a, b; 1942 a, b, c; 1943 a, b; 1950; 1955; 1956), ist gerade die Trockenholztermite *Kalotermes flavicollis* Fabr. besonders gut dafür geeignet, in den einzelnen Speziallaboratorien zur Prüfung von Textilien und anderen Stoffen auf Termitenfestigkeit verwendet zu werden. Auch BECKER (1942 a, b; 1946) berichtet über gute Erfahrungen mit dieser mediterranen Termitenart.

Zweck der nachstehenden Ausführungen ist es, unter Verwertung neuerer Erfahrungen die wichtigsten Grundlagen der Prüftechnik mit *K. flavicollis* zusammenzufassen. Die Ausführungen gelten in ihren Grundlagen neben der Prüfung von Textilien gleichzeitig auch für die Prüfung von Holz und Werkstoffen verschiedenster Art. Es sei ausdrücklich betont, daß die hier nur auf *K. flavicollis* als Versuchstier bezogenen Darlegungen keinerlei prinzipielle Ablehnung von *Reticulitermes lucifugus* Rossi (HERFS, 1950, 1952) zum Ausdruck bringen sollen. Es dürfte im Gegenteil ein Vorteil sein, daß gerade in unseren mitteleuropäischen Laboratorien zwei Methoden mit ökologisch verschiedenen Versuchstieren erarbeitet wurden. Beide Methoden können sich unter Umständen auch gegenseitig ergänzen.

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[1]) Herrn Professor Dr. C. R. BOETTGER zum 70. Geburtstage gewidmet.

In einem zweiten Teil sollen dann später einige Ergebnisse unserer Prüfversuche zur Darstellung gelangen.

DIE WICHTIGSTEN GRUNDLAGEN DER PRÜFTECHNIK MIT *KALOTERMES FLAVICOLLIS* SOWIE IHRE BIOLOGISCHE BEGRÜNDUNG

Die Wahl einer Trockenholztermite als Prüftier

Wegen ihrer besonders großen Überlebensfähigkeit auch bei ungünstigen Bedingungen wie Hunger, geringe rel. Luftfeuchtigkeit etc. sind Trockenholztermiten anerkannt gute Versuchstiere. Da sie im trockenen Holz über keinerlei plastisches Baumaterial verfügen, benötigen sie bei Prüfversuchen nicht die Zugabe von Erde bzw. feuchtem Sand zum Prüfling. Man kann die Versuchstiere daher völlig isoliert in den Prüfhygrostaten halten und hat den Prüfling zu Kontrollzwecken jederzeit frei zur Betrachtung von allen Seiten verfügbar. Ferner kann der Prüfling zur genaueren Kontrolle herausgenommen werden, ohne dabei etwaige Galerien etc. und das feine mikroklimatische Gefüge, auf das empfindliche Arten angewiesen sind, zu zerstören. Zudem sind Trockenholztermiten außerordentlich aggressiv und gehen auf Grund ihres diesbezüglichen Nahrungserwerbes auch härtere Materialien sehr gut an. So hat SCHULTZE-DEWITZ (1957) z. B. nachgewiesen, daß *K. flavicollis* relativ sehr viel mehr Kernholz annimmt als *Reticulitermes lucifugus* Rossi. Prüftechnisch von größter Wichtigkeit ist auch die außerordentlich große Überlebensfähigkeit von *K. flavicollis* im Hungerversuch, da hierdurch Zwangsprüfversuche (GÖẞWALD 1941 a, b) ermöglicht werden.

Das bisher Ausgeführte gilt nach den Berichten amerikanischer Autoren auch für *Cryptotermes brevis*. Gerade diese Trockenholztermite wird für Termitenfestigkeitsprüfungen außerordentlich empfohlen. Sie besitzt offenbar eine ähnliche Aggressivität und hohe physiologische Resistenz wie *K. flavicollis*. BOETTGER (1957) hat daher vor kurzem den Vorschlag gemacht, die Art *Cryptotermes brevis* (Walker) auch in europäischen Laboratorien als Standardprüftermite zu verwenden. So wünschenswert an sich eine Normierung der Prüfmethoden in den Laboratorien der ganzen Welt wäre, so glauben wir doch, daß es sehr schwer ist, die Art *Cr. brevis* in den europäischen Prüfstellen einzuführen. Zwar sollten Materialbeschaffungsschwierigkeiten prinzipiell keine Rolle spielen, jedoch dürfte es nicht immer leicht sein, jederzeit *Cryptotermes* in genügender Anzahl nachzubeschaffen, zumal die Zucht überaus langsam geht und auch die amerikanischen Kollegen das Prüftier prinzipiell dem Freiland entnehmen. Dagegen steht die doch praktisch gleichwertige Trockenholztermite *K. flavicollis* im gesamten Mediterranraum zur Verfügung. Ein Nachteil ist zweifellos die gegenüber *R. lucifugus* geringere Reproduktionskraft der Kolonien von *K. flavicollis*, er läßt sich allerdings bei rationeller Nachzucht mit allem verfügbaren Termitenmaterial, d. h. insbesondere unter Verwendung der bei den Prüfungen nicht brauchbaren Stadien zur Nachzucht (GÖẞWALD, 1943), weitgehend ausgleichen.

Die Auswahl des geeigneten Prüfstadiums

Frühere eigene Untersuchungen (GÖẞWALD, 1941 d, 1942, 1943 b, 1950, 1955, 1956) haben bereits eindeutig ergeben, daß die sog. „Altlarven" von *K. flavicollis* die größte Aggressivität und auch höchste physiologische Resistenz aufweisen. Es handelt sich dabei einerseits um die Larven des 5. Stadiums, andererseits um die sog. Pseudergaten (LÜSCHER, 1952). Insbesondere die Pseudergaten, die aus den L_5 durch progressive Häutungen, aus N_1 durch regressive Häutungen hervorgehen können, spielen eine spezifische Rolle; sie stellen vor allem in älteren Kolonien von *K. flavicollis* eine Art „Zentralstadium" dar, von dem aus alle Entwicklungsmöglichkeiten gegeben sind, das aber auch durch eine nicht festgelegte Zahl von Häutungen über längere Zeit unverändert beibehalten werden kann. Durch diese „Haltestation" in der normal zum imaginalen Geschlechtstier (bzw. zum Soldaten) hin tendierenden Entwicklung wird bei der niederen Termite *K. flavicollis*, die ja keine echte Arbeiterkaste besitzt, erreicht, daß die Kolonien gleichwohl über ein entsprechendes Arbeitspotential verfügen. Zusammen mit den L_5 stellen die Pseudergaten also die *funktionellen* Arbeiter dar. Wegen der schwankenden Größenverhältnisse der Kopfkapselbreite (sie folgt einer flachen Binomialkurve und überschneidet sich mit den benachbarten Stadien) ist eine genaue Festlegung des Stadiums bei den Larven nicht immer möglich. Erschwert wird dies noch dadurch, daß in Abhängigkeit von der Koloniegröße und offenbar auch vom Herkunftsort, die Größe der Tiere weiter modifiziert ist. So haben jüngere Kolonien durchschnittlich kleinere Altlarven und Nymphen als ältere, und Kolonien aus Banyuls (SW-Frankreich) lagen durchschnittlich in ihrer Larvengröße unter der von Kolonien aus dem Raum Neapel. Unter Anlehnung an LÜSCHER (1952) verstehen wir unter L_5 Tiere mit einer Kopfkapselbreite von 0,93—1,10 mm, unter Pseudergaten solche mit einer zwischen 1,10—1,18 mm schwankenden Kopfbreite. Zur Sicherung der bereits früher gemachten Feststellung, daß Altlarven die größte Aggressivität besitzen, wurden in unserem Isotopenlaboratorium unter unserer Anleitung von Fräulein J. Bertho, Paris, Schülerin von Professor Dr. P. P. Grassé, in den vergangenen Monaten radiobiologische Untersuchungen an *K. flavicollis* durchgeführt. Ziel der Untersuchung war es, mit Hilfe von P^{32} herauszufinden, welche Stadien über-

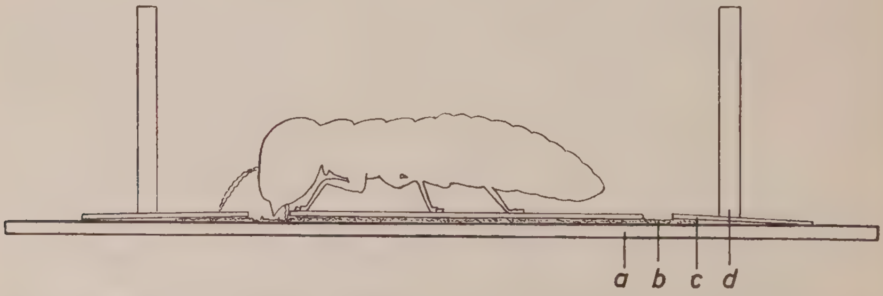

Abb. 1: Anordnung zur peroralen Markierung von *K. flavicollis* unter weitgehender Vermeidung äußerlicher Kontamination: a = Glasunterlage; b = mit radioaktiver Phosphatlösung getränktes Filterpapier; c = termitenfeste Kunststoff-Folie mit eingestanzten Löchern; d = Glasring zur Einzwingerung des Versuchstieres. Zeichnung T. Fenzl.

haupt aktiv Nahrung aufnehmen und wieviel sie fressen. Methodisch wurde dabei wie folgt verfahren: Filterpapier wurde mit einer radioaktiven Phosphatlösung (spezifische Aktivität 1 mC/cm^3) getränkt und nach Trocknung isolierten Gruppen gleichartiger *Kalotermes* Stadien zum Befressen angeboten. Die Tiere wurden in bestimmten Zeitabständen gemessen und der Grad ihrer Radioaktivität wurde mit der Menge der aufgenommenen Nahrung identifiziert. Um eine Kontamination am aktiven Filterpapier zu verhindern, wurde das Filter mit einer termitenfesten Kunststoff-Folie überdeckt, die an einigen Stellen, entsprechend dem Durchmesser des *Kalotermes*kopfes, durchlocht war (Abb. 1). Die Tiere haben sofort durch die gebotenen Löcher das Filterpapier angenommen.

Die radiobiologischen Untersuchungen führten zu dem Ergebnis, daß die Larven des 1. und 2. Stadiums überhaupt nicht selbständig Nahrung aufnehmen, dies gilt gleichermaßen für die Soldaten, Präimaginalstadien (= kurzes Zwischenstadium zwischen N_2 und endgültiger Imago) und Geflügelten. Am aktivsten sind die Larven des 3., 4. und 5. Stadiums sowie die Pseudergaten. Auf Grund ihrer Größe nehmen jedoch die beiden letztgenannten Stadien die höchste Radioaktivität auf. Ebenfalls die N_1 und auch N_2 sind noch überaus aggressiv, nehmen jedoch nur nach einer Präadaptionszeit von etwa 2 Tagen Nahrung auf, was auch z. T. für die Pseudergaten gilt. Offenbar sind Nymphen und manchmal auch Pseudergaten mehr Nahrungsempfänger als aktive Fourageure und benötigen daher eine gewisse Zeit zur Umstellung auf selbständige Nahrungsaufnahme. Da N_1 und auch N_2 aber auch überaus aktiv sind, kann ohne weiteres bei Mangel an „Altlarven" auf Nymphen, vor allem des 1. Stadiums (ganz kurze, anliegende Flügelstummel) zu Prüfzwecken zurückgegriffen werden.

Die benötigte Anzahl von Prüftieren

Wie bekannt ist, werden von uns für die 1. Prüfung 30 „Altlarven" vorgeschlagen (GÖSSWALD 1942, 1955, 1956). Die Begründung, daß erst 30 Tiere eine genügend große Gruppe darstellen, erfolgte seinerzeit auf Grund biologischer Erfahrungen. Es ist vielfach der Vorschlag gemacht worden, diese Anzahl zum Zwecke der Ersparnis von Versuchstieren zu reduzieren (etwa auf 10 Tiere) und auch auf die tägliche Ergänzung zur vollen Zahl zu verzichten. Es muß jedoch (mit Ausnahme von Vortesten auf insektizide Wirkung) aus folgenden Gründen weiterhin an dieser Zahl festgehalten werden:

a) Die erwähnten radiobiologischen Untersuchungen ergaben die interessante Erscheinung, daß die sich häutenden Termiten 2 Tage vor und 2 Tage nach der Häutung keinerlei Nahrung aufnehmen. Sie fressen weder selbständig noch werden sie von anderen Tieren gefüttert. Damit fällt jedes sich häutende Tier für 4 Tage hinsichtlich seiner Prüfwirkung völlig aus. Nimmt man etwa nur 10 Tiere, von denen sich im Verlaufe der Prüfung 50% häuten, so ist die Anzahl der angreifenden Prüftiere zu gering. Bei größerer Zahl von Versuchstieren ist viel leichter mit einer gleichmäßigen Angriffswirkung zu rechnen. Auch wenn sich ein Teil der Tiere häutet, so bleibt doch jederzeit eine genügende Zahl aggressiver Prüftiere zurück.

b) Die Zusammenfassung einer Gruppe von „Altlarven" zu Prüfzwecken führt auf Grund des Wegfallens der Hemmwirkung der funktionellen Geschlechtstiere zur Ausbildung von Ersatzgeschlechtstieren (EG). In der sensiblen Phase befindliche Tiere werden dann zur Entwicklung zu EG induziert. Die „Kompetenz" zur EG-Entwicklung steht in Abhängigkeit vom Abstand von der letzten Häutung (LÜSCHER, 1952). Je kürzer der Zeitraum seit der letzten Larvenhäutung ist, desto leichter erfolgt die Induktion zur EG-Häutung. Wenngleich die Häutung zum EG sehr viel rascher stattfindet (im Mittel nach 39 Tagen) als die nächstfolgende normale Larvenhäutung, so muß doch bei Verwendung von bereits länger „entweiselten" Larven mit EG-Häutungen im Verlaufe der Prüfzeit gerechnet werden. Es ist nun zunächst noch nichts darüber bekannt, ob zur EG-Entwicklung induzierte Larven in normaler Weise Futter aufnehmen oder ob sie bei Vorhandensein anderer Larven eventuell Futterempfänger darstellen. Diesbezügliche radiobiologische Untersuchungen sind im Gange. Es dürfte jedenfalls auch hier die verlangte Zahl von 30 Prüftermiten mit Sicherheit dazu führen, daß ein gleichmäßiger Prüfeffekt gewährleistet ist.

Im einzelnen ist es außerordentlich schwer, immer ganz streng vergleichbare Prüfgruppen zusammenzustellen. Man müßte, um dieses Ziel zu erreichen, das individuelle Häutungsalter eines jeden Einzeltieres exakt kennen. Die Verwendung der doch immerhin schon größeren Zahl von 30 Tieren führt aber rein statistisch zu einem Ausgleich der physiologischen Verschiedenheiten und ermöglicht somit die Durchführung von *jederzeit reproduzierbaren Prüfungen!* Daraus folgert gleichzeitig auch die Berechtigung unserer Forderung des täglichen Ersatzes toter und schwacher Tiere durch lebenskräftige „Altlarven". Da die Ausfälle ganz verschiedenartig bedingt sein können (in länger verweisten Gruppen z. B. durch die Elimination überzähler EG-Tiere), wird auf diese Weise durch die tägliche Ergänzung zur vollen Zahl zum statistischen Ausgleich und damit zur Reproduzierbarkeit der Prüfung beigetragen. Die Erläuterungen dürfen dahingehend zusammengefaßt werden, daß an der Mindestzahl von 30 Tieren in den Prüfgruppen festgehalten werden muß.

Die Mindestprüfzeit von 21 Tagen.

Termitenfestigkeitsprüfungen dürfen auch im Fraßzwangsversuch nicht zu kurz angesetzt sein. Insbesondere dann, wenn die Prüflinge abschreckend wirken oder keinen natürlichen Nahrungsanreiz bieten, werden sie in den ersten Tagen überhaupt nicht oder nur sehr verzögert angenommen. Erst wenn die Tiere einen Teil ihrer Reserven durch das Hungern verbraucht haben, beginnt sich ihre Angriffslust zu steigern. Dies tritt erfahrungsgemäß nach einer Reihe von Tagen ein, die Prüfung darf daher unter keinen Umständen zu früh beendet werden, weil dadurch das Ergebnis einer nur scheinbaren Termitenfestigkeit erzielt werden könnte. Aber auch bei sehr gerne angenommenen Materialien zeigt sich häufig eine Verzögerung von 2 Tagen, ehe Nahrung angenommen wird. Dies gilt immer für N_1, die nach unseren radiobiologischen Ergebnissen bei Entfernung aus dem normalen Kolonieverband erst nach 2 Tagen mit der selbständigen Nahrungsaufnahme beginnen. Alle diese Störfaktoren werden durch die von uns als genügend lange angesehene Prüfzeit von 21 Tagen völlig ausgeglichen. Zugleich ist diese Zeit auch nicht zu lange angesetzt, es können im Interesse der Industrie doch relativ rasch Ergebnisse erzielt werden.

DIE NORMALMETHODIK DER TEXTILPRÜFUNG MIT *KALOTERMES FLAVICOLLIS*

Auswahl der Textilien und Anordnung der Prüfung in Abhängigkeit vom Zweck der Prüfung.

Da die einzelnen Textilien, wie bereits in der Einleitung erwähnt wurde, auch unbehandelt sehr verschieden stark von Termiten angegriffen werden, ergibt sich zwangsläufig die Notwendigkeit zur Feststellung der spezifischen Wirkung eines Imprägniermittels genormte Trägerstoffe zu verwenden, wie ja auch für die Prüfung von Holzschutzmitteln eine bestimmte Holzart, nämlich *Pinus silvestris* empfohlen wird. Man wählt für die Untersuchung von Imprägniermitteln einen von den Termiten leicht angreifbaren Standardstoff für jede Stoffkategorie. Sehr leicht angreifbare Stoffe sind z. B. Viskose-Stoffe und Kupferkunstseiden. Zur Feststellung der Widerstandsfähigkeit an vollsynthetischen Stoffen, die bereits im nicht imprägnierten Zustand auf Grund der Termitenfestigkeit ihrer Rohfasern vor Fraß geschützt sein sollen, wird eine Qualität als Standardstoff verwendet, die unter Bedingungen, welche für die Versuchstiere optimal sind, gerade noch schwach beschädigt werden kann, um auf diese Weise die Angriffsbereitschaft der Versuchstiere zu überwachen (GÖSSWALD 1950). Ein solcher Stoff ist z. B. eine bestimmte Segeltuchqualität der Firma Dr. Quehl, Speyer.

Klimatisierungen und Prüfverlauf

Bereits durch die Anwendung der für Tropeninsekten in Frage kommenden Prüfmethoden ist die Klimatisierung der Materialien eingeschlossen. Im einzelnen verlaufen unsere Mittelprüfversuche folgendermaßen:

Temperatuur 26° C konstant im Zuchtraum bzw. für Mittelprüfungen im Brutofen. Relative Luftfeuchtigkeit 98%, reguliert durch K_2SO_4 und H_2O bzw. in den Zuchten mittels feuchtem Gipsbelag. Versuchsschalen: Münchener Hygrostaten, welche aus folgenden Stücken bestehen: Bodenstück mit Kaliumsulfatbrei; der obere innere Rand wird mit Vaseline bestrichen, um ein Hochsteigen der Salzlösung (mit Bodenkörper von K_2SO_4) zu verhindern. Ein einlegbarer Glasring wird mit feinster Messing oder Phosphorbronzegaze bespannt. Der Rillendeckel paßt auf den Einlegring. Die Luftzufuhr zwischen Einlegring und Bodenstück genügt; die Feuchtigkeit wird vom K_2SO_4-Brei her durch das Metallgewebe nach oben reguliert.

Die Versuchsschalen werden zweckmäßig in Holzkästchen gestellt, so daß sie bequem in größerer Zahl aus dem Brutofen entnommen und hierhin zurückgesetzt werden können.

Prüfverlauf: Die erste Prüfung ist eine Ausleseprüfung; sie wird mit 30 Altlarven durchgeführt; bei der täglichen Kontrolle werden Fraßschäden verzeichnet, gestorbene oder schwach gewordene Termiten werden aus der Zucht durch gesunde ergänzt, so daß stets die volle Zahl von Versuchstieren drei

Wochen lang einwirken kann. Bereits früher eindeutig befressene Prüflinge können schon vor Ablauf der sonst drei Wochen dauernden Prüfung ausscheiden. Es wird unterschieden zwischen undeutlichem, schwachem und starkem Schabefraß sowie Kanten- und Lochfraß.

Die zweite Prüfung umfaßt den gleichen Prüfling, wenn dieser bisher standgehalten hat, diesmal mit 150 Altlarven. In der Regel sind die Beschädigungen nach Abschluß dieser mit verstärkter Bestückung durchgeführten Prüfung deutlich stärker. Hat aber gleichwohl nach drei Wochen bei täglicher Ergänzung schwacher und toter Termiten das Prüfobjekt den Angriffen standgehalten, so wird das bisher fabrikneu geprüfte Material nunmehr einer Gebrauchsechtheitsprüfung unterzogen, d. h. je nach Verwendungszweck klimatisiert (a) Wüsten- und b) Urwaldklima, verkürzte Einwirkungszeit zur Beschleunigung des Prüfergebnisses, dafür Temperaturerhöhung: vier Wochen a) 60° C und 98% rel. Luftfeuchtigkeit, b) 60° C und 40% rel. Luftfeuchtigkeit), oder bei Wäschestücken mehrmals mit Waschpulver gewaschen, dazwischen jedesmal getrocknet, gebügelt etc., dazu wird ein künstlicher Beschmutzungsprozeß durchgeführt durch Anreicherung des Stoffes mit Diastase, andere Materialien werden gewässert, in Süßwasser oder salzhaltigem Wasser, gesonnt usw. Die Klimatisierung wurde in Anlehnung an die DN-Normen für trockenheiße und feuchtwarme Prüfräume zunächst 3 Wochen, im Max. bis 6 Monate durchgeführt. Allgemeine Richtlinien bestehen bisher, im Hinblick auf die Verschiedenartigkeit des Materials und Verwendungszweckes nicht.

Die dritte Prüfung setzt nach der Klimatisierung bzw. nach der Wäsche, Auslaugung, Bestrahlung usw. (Einzelheiten vgl. meine früheren Veröffentlichungen, sowie BECKER 1946) wieder mit 30 Termiten ein unter gleichen Bedingungen wie sie bei der ersten Prüfung erwähnt wurden. Wenn in diesem Gebrauchszustand wieder keine Fraßschäden zu bemerken sind, folgt die endgültige vierte Prüfung mit 150 Termiten, nach deren Absolvierung der Prüfling endlich das Prädikat „termitenfest" erhält, sofern keine Beschädigung festzustellen ist.

Eine modifizierte Prüfanordnung

Da es bei Textilproben je nach Gewebeart oft nicht ganz leicht ist, ganz glatte Ränder zu schaffen, suchten wir nach einer einfachen und billigen Möglichkeit, die rechteckigen Proben am Rande einzufassen. Erfahrungsgemäß ist nämlich an verfransten Rändern oft nur schwer zu entscheiden, ob ein Randfraß vorliegt oder nicht. Man schaltet daher vorteilhafterweise die Möglichkeit eines Randfraßes völlig aus und läßt nur Flächen- und Lochfraß zu. Als besonders günstig erwiesen sich Ganzmetall-Diawechselrähmchen (Fabrikat CENEI). Man läßt die beiden Gläser im Rähmchen und spannt auf einer Außenseite die Textilprobe straff zwischen Metallrahmen und Glas ein. Entsprechend der Fenstergröße 24 × 36 mm (Normal-Leicaformat) wird den Termiten also der Prüfling angeboten. Das Objekt wird mit der Textilseite nach unten gelegt, wobei das Auflegen der Metallränder auf ganz dünne Glasstückchen (Objektträgerdicke) vorteilhaft ist. Die stark ausgeprägte Bereitschaft zu thigmotaktischen Reaktionen führt die Tiere dann an das zu prüfende Textilgewebe heran (Abb. 2 oben). Neben dem zunächst angestrebten Zweck einer Randeinfassung bieten die Metall-Wechselrähmchen

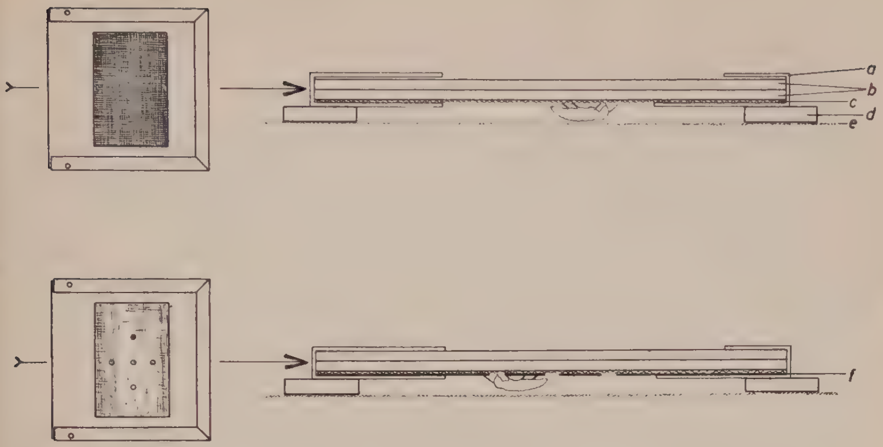

Abb. 2: Verwendung von Metall-Glas-Diapositivrähmchen zur Prüfung von Textilien auf Termitenfestigkeit. Links jeweils Aufsichtsbild, rechts Schnitt durch die Versuchsanordnung. Oben: Normalmethode. Unten: Modifizierte Methode mit durchlochter Kunststoff-Folie. a = Metallrähmchen; b = Glasplättchen; c = Textilprobe, eingespannt zwischen Außenseite des Glasplättchens und Metallrahmen; d = Glasstückchen zur seitlichen Auflage des Rähmchens; e = Phosphorbronzegaze der Hygrostatschale; f = gelochte, termitenfeste Kunststoff-Folie als Auslöseschema (vgl. Text). Zeichnung T. Fenzl.

noch weitere Vorteile: Sie ermöglichen eine sehr schöne Kontrolle im durchfallenden Licht bei Projektion an eine Wand. Vor allem leichter und geringster Schabefraß, den man bei Auflichtbeleuchtung oftmals nur sehr schwer feststellen kann, läßt sich auf diese Weise hervorragend feststellen. Es ist eine Kleinigkeit, die Proben täglich kurz herauszunehmen und im Projektionsapparat zu durchleuchten. Ein weiterer Vorteil liegt darin, daß man im Kontaktverfahren bzw. mit dem Vergrößerungsapparat direkte Protokollphotogramme aufnehmen kann, durch die eine objektive Prüfberichterstattung sehr erleichtert wird. Diese einfach zu handhabende Methode könnte durchaus als Norm-Methode vorgeschlagen werden, zumal sie wegen der nur einseitigen Angriffsmöglichkeit (die andere Seite ist ja durch das Glas abgedeckt) rasch zu Ergebnissen führt.

Die Lochmethode, eine verschärfte Prüfmethode für Spezialzwecke

Im Verlaufe unserer Untersuchungen haben wir noch eine Spezialmethode entwickelt, die auf verhaltensphysiologischen Grundlagen beruht und sowohl biologisches als auch prüftechnisches Interesse verdient. Es wurde bereits geschildert, daß bei Aktivierung der Tiere in radiobiologischen Untersuchungen zur Vermeidung einer Kontamination mit dem radioaktiven Präparat das Filterpapier nur durch eine gelochte Plastikfolie angeboten wird. In gleicher Weise wurden von uns auch Textilien geprüft. Die Proben werden dazu in die erwähnten Ganzmetall-Wechselrähmchen eingespannt, sind aber nach außen hin mit einer dünnen Folie aus einem termitenfesten Kunststoff überdeckt. Die Kunststoff-Folie hat 5 Löcher mit einem Durchmesser von 1,2 mm. Nur durch diese Löcher können somit die

Tiere das Textilmaterial erreichen (Abb. 2 unten). Bei solchen Versuchen stellte sich folgendes Ergebnis heraus: Auch Textilien, die bei Durchführung der normalen Prüfmethodik (1. Prüfung — Klimatisierung — 2. verschärfte Endprüfung) als völlig termitenfest ohne jeden Fraßschaden verblieben waren, wurden im Laufe weniger Tage durchgefressen. Es zeigten sich bereits nach 2 Tagen tiefgehende Schabestellen, der Befraß wurde dann im Bereich der Löcher kontinuierlich weitergeführt. Zunächst erscheint dieser Befund verblüffend und mit dem Resultat der Normalprüfung nicht vereinbar. Sobald man jedoch mit den Methoden der modernen Verhaltensphysiologie das Ergebnis analysiert, ergibt sich Folgendes: Der Durchmesser der Löcher in der Folie entspricht weitgehend dem Durchmesser des Kopfes der „Altlarven" von *K. flavicollis.* Damit entspricht er auch gleichzeitig dem Durchmesser der normalen Fraßgänge der verwendeten Termitenart. Da die Folie eine Dicke von 0,1 mm besitzt, stellt sie für Termiten an den Löchern einen kreisrunden Rand dar, der genau dem Rand eines begonnenen Fraßganges etwa im Holz entspricht. Kreisrunde Löcher bzw. Ränder dieser Größe dürften aber auf Grund unserer Versuche für die Termiten ein angeborenes Auslöseschema für das Instinktverhalten „Fraßgang-Nagen" darstellen. Entsprechend ihrer sozialen Verhaltensweisen bemühen sich die Tiere um Weiterführung des angefangenen „Fraßganges". Es ist uns nun sehr wohl bekannt, daß Termiten bei der Anlage von Gängen auch Materialien zu durchnagen vermögen, die normalerweise nicht als Nahrung dienen. Damit wird es verständlich, daß auch solche Materialien bei dieser Prüfanordnung durchnagt werden, die sich sonst als resistent erweisen. Wegen dieser verhaltensphysiologischen Bedingtheit eines Lochfraßes unter der dargelegten Prüfanordnung lehnen wir diese Prüfmethode als zu hart ab. Bei ihrer Anwendung dürfte es außerordentlich schwer sein, überhaupt noch termitenfeste Materialien zu finden. Dagegen erscheint die Methode für Spezialfälle überaus interessant, wie zum Beispiel dann, wenn ein Präparat auf „Repellentwirkung" getestet werden soll. Es kann dann etwa entschieden werden, ob die Repellent-Wirkung stärker ist als die Wirkung des Loches als Auslöseschema für die Weiterführung des „Lochfraßes". Auch in solchen Fällen, in denen die Materialien auf Grund ihrer Oberflächenbeschaffenheit entweder zu hart oder zu glatt für den Termitenangriff sein sollen, ermöglicht die Lochmethode eine exakte Überprüfung dieser erwünschten Oberflächenwirkung. Es wird daher der Vorschlag gemacht, die Lochprüfmethode für derartige Spezialzwecke zuzulassen, als Normalprüfmethode dagegen als zu streng abzulehnen.

Eine Überprüfung der Methode bei *Reticulitermes lucifugus* (Einlegen in die Zuchtaquarien, also kein Zwangsversuch) ergab bisher keinen Lochfraß, die Wirkung des Loches als Auslöseschema muß jedoch für diese Art in Zwangsversuchen noch näher überprüft werden.

SUMMARY

LABORATORY EXPERIMENTS ON THE RESISTANCE OF VARIOUS MATERIALS, ESPECIALLY TEXTILES, TO KALOTERMES FLAVICOLLIS *FABR.*

Experiments were made over a period of two years on the resistance of various materials, especially textiles, to termites. The dry wood termite, *Kalotermes flavicollis* Fabr. was used as a test animal, as this insect is resistant to environmental conditions and it readily attacks hard materials. Quantitative radio-biological investigations showed that fifth-instar larvae and "Pseudergates", which both feed actively, are good test animals. After moulting, larvae do not feed for 4 days. It is advisable therefore to use large batches (30 larvae in each) to ensure that some are trying to feed at any time. Experiments should last 21 days at least. The method used in testing the effects of *K. flavicollis* on textiles, and the way the insect is acclimatised, are described. A useful new technique for these tests consists of pulling the textile into the metal frame of a projector-slide. Termites can gnaw through materials otherwise resistant when they are covered by a thin perforated film of termite-proof material. If the perforations are of the same diameter as the termite's head they serve as innate releasing mechanisms for "food-tunnel gnawing". This method should only be used in special cases, e.g. testing for repellency, surface hardness, etc.

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FEEDING AND EXCRETION BY THE PEA APHID, *ACYRTHOSIPHON PISUM* (HARR.) (HOMOPTERA: APHIDIDAE), REARED ON DIFFERENT VARIETIES OF PEAS

BY

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The amount of honeydew excreted by the pea aphid, *Acyrthosiphon pisum* (Harr.), reared singly and in groups on susceptible resistant varieties of peas, *Pisum sativum* L., grown under greenhouse conditions, was measured. Excretion by isolated aphids was interrupted at moulting for mean periods of 12 and 16 hr, and between moults and in the adult stage for mean periods of 3 to 15 hr, although in many cases the aphids remained in the feeding position. Droplet volume, frequency, and rate of excretion were generally proportional to the susceptibility of the host variety on which the aphids were feeding. The rate of feeding on susceptible varieties was higher and contributed therefore to the faster rate of aphid growth; a greater proportion of the material ingested was excreted and a lesser proportion assimilated than on resistant varieties. Honeydew from aphids feeding on susceptible varieties usually contained a slightly higher concentration of free amino acids and amides.

Early field observations on the resistance of varieties of peas, *Pisum sativum* L., to the pea aphid, *Acyrthosiphon pisum* (Harr.), by SEARLS (1935) and MALTAIS (1937, 1951) indicated that varieties differed in degrees of infestation and in the rate of aphid population increase. Experiments under greenhouse conditions by HARRINGTON (1941), HARRINGTON, SEARLS, BRINK & EISENHART (1943) and CARTIER (1959, and unpublished data) showed that the rates of aphid growth and reproduction were significantly lower on resistant varieties. These results suggested nutritional differences as contributing factors of resistance, and studies by MALTAIS & AUCLAIR (1957) and AUCLAIR, MALTAIS & CARTIER (1957) showed that the sugar-nitrogen ratio was 23.4 to 63.7 per cent higher in the resistant varieties Laurier, Champion of England and Melting Sugar than in the susceptible varieties Perfection, Daisy and Lincoln; these susceptible varieties generally contained a higher concentration of free and total amino acids at the stages of growth and in the parts of the plants usually infested in the field. It appeared therefore that aphids on resistant varieties fed on plant material of lower nutritional value. Whether the aphids were able to correct this relative deficiency by a higher rate of feeding on resistant varieties remained a possibility. In order to estimate indirectly the rate of aphid feeding, a quantitative method for measuring their frequency and rate of excretion was developed (AUCLAIR 1957, 1958a). This is a report on the rate of feeding and excretion of the pea aphid as affected by varieties of peas.

MATERIALS AND METHODS

The greenhouse conditions together with the growing and preparation of the pea plants prior to honeydew collection were described by AUCLAIR (1958a). Apterous virginoparae, uniform as to stock, (R1, CARTIER, 1957), and age i.e., all specimens used in a given experiment were born during the same 24-hour period, were used throughout.

Experiments with isolated aphids. Four circular turning tables, one-foot in diameter and powered by a standard electric tork-clock, were set up in a room at 70° F. (± 2), 50 per cent (± 8) relative humidity, and with fluorescent and incandescent lighting (about 500-footcandles intensity) from 8 : 00 a.m. to 11 : 00 p.m. One five-week old potted pea plant of variety Perfection or Onward and bearing one aphid was suspended horizontally about one inch above each table, the latter being covered with a filter paper disc treated with a pH indicator. The discs were changed daily. Honeydew droplets excreted by the aphids became immediately visible as blue spots upon hitting the paper. With this timing device, similar to that used by SMITH (1937), hourly records were obtained on the frequency of excretion and the lengths and cycles of the excretory periods. Visual observations were also made about every hour between 9 : 00 a.m. and 5 : 00 p.m. with a magnifying lens (8 ×) on moulting and on the aphid movements and feeding sites on the host. Ten series of experiments with 40 isolated aphids were made.

Experiments with aphid populations. Six experiments were conducted under greenhouse conditions with three to six varieties and eight to twelve plant replicates per variety. The plants at the beginning of experiments A, B, C, D, E and F (Table II) were respectively 24, 21, 25, 26, 40 and 50 days old, averaged 8, 7, 8, 9, 11 and 17 internodes, the terminal bud being counted as the first internode, and measured 13 to 37 inches for the varieties Melting Sugar, Champion of England-1 and Laurier-12, and 5 to 18 inches for Perfection, Lincoln, Daisy and Onward. Each plant was infested with ten to twelve aphids (for instars used, see Table II), some 3000 aphids being used for all experiments. Measurements of honeydew droplet volume, optical density of honeydew spots, and frequency and rate of excretion were carried out as described by AUCLAIR (1958a).

In addition, in experiment D lasting 71 hr, the rate of ingestion was determined indirectly by adding the body weight increase to the weight of the material lost by evaporation, excretion and moulting. Aphids were weighed at 0, 22, 47 and 71 hr to determine weight increase. The rate of body evaporation and possibly slight losses through respiratory gas exchanges were estimated by weighing groups of third, fourth instar nymphs, and non-bearing adults, before fasting, in small loose-mesh nylon bags in the greenhouse in close vicinity of pea plants and weighing again after 5 hr. The rate of evaporation per hr was then determined and assumed to be constant throughout each instar. Exuviae were also weighed. The specific gravity of honeydew was determined by weighing microliter volumes of fresh honeydew and dividing the weight obtained by that of equal volumes of distilled water. Weighing of water and honeydew on small pieces of filter paper of known weight was carried out with a Roller-Smith microtorsion balance (sensitivity of

2 micrograms). The amount lost through evaporation during weighing, being similar for water and honeydew, did not therefore represent a source of error in establishing the ratio.

RESULTS AND DISCUSSION

With isolated aphids. Results indicated that honeydew excretion was not a continuous process. This was also reported by SMITH (1937) on the mealy plum aphid, *Hyalopterus pruni* (Geoff.). On Perfection, Table I, series 1 and 2, excretion ceased during an average of 12 hr at the time of moulting, although the actual shedding of the exuviae took less than one hour. Excretion by third and fourth instar nymphs was also interrupted between moults for mean periods of 3.5 hr, the interruption being longer (10.5 hr) when it was accompanied by a visible change in the aphid feeding position. Non-excretory periods (one to 9 hr) were also observed with adults (Series 1). Non-excretory periods alternated with excretory periods of one to 22 hr duration, during which the frequency varied from 0.4 to 2.1 droplets per hr. BECK, EDWARDS & MEDLER (1958) studying the feeding and nutrition of fifth-instar nymphs of the milkweed bug, *Oncopeltus fasciatus* (Dallas), observed that feeding occurred in cycles averaging about 22 hr separated by inactive intervals of 2 to 6 hr. No feeding occurred during the 48 hr preceding the imaginal ecdysis. In the present work, the absence of excretion during moulting was to be expected since the aphid stops feeding at that time. However, no satisfactory explanation can be given for those interruptions in excretion between moults, and during the adult stage, since in the majority of cases, the aphids under observation remained settled on the plant with their labial sheaths in feeding position. Whether the stylets were retracted into the sheath or were probing into the tissues in search of other suitable areas was not ascertained. No correlation could be established between excretion and the photoperiods. Aphids in most cases placed the tip of their labial sheath on the main leaf veins or their ramifications. It is known (MALTAIS, unpublished; MITTLER's review, 1954; SKOTLAND & HAGEDORN, 1955) that the stylets of the pea aphid usually penetrate the plant to the phloem sievetubes to feed mainly on the phloem sap. In a few cases it was observed in the present work that the tip of the labial sheath of settled aphids was not placed on main leaf veins or their visible ramifications and this was accompanied by an appreciable decrease in the frequency of excretion per hr.

Aphids feeding on the resistant variety Onward (Table I) had shorter excretory periods during which the frequency of excretion was lower. Their non-excretory periods were longer and the aphids changed their feeding sites more than twice as often as those on susceptible Perfection (e.g. 30 against 13 times in 60 hr).

With aphid populations. Results of the six experiments (Table II) showed that frequency and rate of excretion were highest on Perfection, high on Lincoln, significantly lower on Champion of England-1 and Laurier-12, and lowest on Melting Sugar, Daisy and Onward. The frequency and rate of excretion for varieties Perfection and Lincoln differed (Student's *t* test) at the 5 per cent

TABLE I

Excretion by single pea aphids feeding on two varieties of peas
(Standard Deviations are in parentheses)

	Frequency of excretion			Non-excretory periods (hr)			
Aphid Stage	Excretory periods (hr) Mean*	Droplets/hr during continuous excretion Mean	Droplets/hr based on periods of a few days Mean	With moulting occurring Mean	Excluding moulting Mean	With visible change in aphid feeding position Mean	Nos of aphid feeding positions in 60 hr
	On susceptible variety Perfection (series 1)						
Third and fourth instar	6 (4.6)	1.3 (0.4)	0.4	12 (4.5)	3.5 (1.5)	10.5 (6.7)	
Adults	8.3 (5.1)	1.4 (0.4)	0.9	—	3.7 (1.9)	4.2 (2.1)	
	(series 2)						
Third instar to adult	6.3 (3.8)	1.0 (0.2)	0.6	12 (4.9)	5.8 (3.5)	11.3 (4.7)	13
	On resistant variety Onward (series 2)						
Third instar to adult	2.5 (1.0)	0.9 (0.2)	0.2	16 (8.6)	10.9 (10.4)	15.0 (10.5)	30

* Periods of one hour or less were omitted when calculating the mean.

level of significance from that recorded for Champion of England-1 and Laurier-12, and at the one per cent level from Melting Sugar, Daisy, and Onward, in most cases of the six experiments. Concurrent experiments on the growth of separate aphid (R1) populations made under greenhouse conditions by our colleague CARTIER (unpublished) showed the mean weight of aphids reared on varieties to be: on Perfection, 4.18 mg; Lincoln, 3.47; Champion of England-1, 3.01; Laurier-12, 2.77; Daisy, 2.69; Melting Sugar, 2.46; and Onward, 1.98. A weight difference of 0.38 mg was significant at the five per cent level. The above varieties are listed in order of increasing resistance as related to aphid growth and excretion. For some as yet little known reasons, Daisy recognized as a susceptible variety in the field (MALTAIS, 1937; MALTAIS & AUCLAIR, 1957) showed resistance under greenhouse conditions.

The reduction in excretion was observed in all instars and adults and that in rate was generally accomplished by the production of smaller droplets at a lower frequency. This lower frequency partly resulted from the facts that aphids, especially adults, changed their feeding sites more often, their frequency of excretion per hour was lower and their non-excretory periods longer. This is also supported by the results with individual aphids on Onward (Table I). The excretion values for experiment F were generally lower than those for all other experiments probably because the plants on which the aphids were feeding in F were the oldest, having reached the podding stage when the test began. Daily records indicated that for the first 12 hr of each experiment, excretion rates on resistant varieties were lowest, although the mean weights of aphids on all varieties were then comparable. This indicated that the reduction in excretion preceded that in growth. Optical density measurements of honeydew spots (Table II) indicated generally that aphids on susceptible varieties Perfection and Lincoln excreted a honeydew containing a higher concentration of free amino acids and amides than that excreted on the resistant varieties excepting Daisy. This was confirmed by quantitative two-dimensional paper chromatography of honeydew. Results on the concentration of sugars in honeydew were inconsistent.

The rates of ingestion ranged from 1.4 to twice as high as those of excretion for all varieties (see ratios, Table III); and were approximately twice as high on susceptible Perfection as on resistant Daisy, Melting Sugar, Laurier-12 and

TABLE II

Excretion by pea aphid populations feeding on varieties grown under greenhouse conditions. Summary of six experiments. St. Jean, Que., 1956—58.

Experiment	Pea varieties	Plant height (inches)	Aphid instar	Mean droplet volume (μls)	Mean frequency (droplets per aphid per 10 hr)	Mean rate (μls per aphid per 10 hr)	Mean optical density of honeydew spots (from about 4000 readings)		Per cent honeydew spots with optical density above 100 amino acids
							animo acids	sugars	
A	Perfection	7	First to second	—	3.4	—	100	—	57
	Champion of England-1	18		—	2.3	—	92	—	52
	Laurier-12	21		—	2.1	—	84	—	22
	Melting Sugar	19		—	2.0	—	76	—	12
	Onward	6		—	1.8	—	94	—	34
B	Perfection	6	Fourth to bearing adult	0.13	5.7	0.74	83	80	38
	Champion of England-1	15		0.11	3.6	0.40	69	84	16
	Laurier-12	18		0.14	3.3	0.46	75	81	23
	Melting Sugar	15		0.13	2.4	0.31	79	76	23
	Onward	5		0.10	2.9	0.29	83	77	33
C	Perfection	8	Fourth to bearing adult	0.17	3.2	0.54	80	—	37
	Laurier-12	22		0.15	2.7	0.41	61	—	26
	Onward	7		0.14	1.9	0.27	46	—	24
D	Perfection	8	Third to adult	0.19	5.4	1.03	117	27	77
	Lincoln	6		0.14	4.6	0.64	104	24	63
	Champion of England-1	18		0.11	4.2	0.46	94	39	54
	Laurier-12	22		0.14	3.1	0.43	83	39	39
	Melting Sugar	18		0.12	3.5	0.42	83	35	38
	Daisy	6		0.13	2.6	0.34	102	38	59
E	Perfection	8	Third to adult	0.17	5.5	0.94	111	41	72
	Lincoln	6		0.17	4.9	0.83	106	37	71
	Champion of England-1	13		0.16	3.4	0.54	98	39	58
	Laurier-12	20		0.17	2.9	0.49	103	42	59
	Daisy	6		0.17	2.8	0.48	133	31	88
	Melting Sugar	16		0.15	2.9	0.44	111	26	71
F	Perfection	18	Second to adult	0.14	1.8	0.25	73	—	18
	Onward	19		0.12	1.5	0.18	50	—	18
	Daisy	12		0.09	1.8	0.16	73	—	29
	Melting Sugar	37		0.11	1.4	0.15	43	—	5

TABLE III

Mean rates of ingestion and assimilation, per cent material excreted and assimilated and ingestion/excretion ratios as determined from mean body weight increase, weight losses by evaporation, exuviae and excretion for pea aphids reared on six varieties during 71hr. Experiment D of Table II.

Pea variety	Final weight	Body weight increase**	Body evaporation	Approx. weight of 2 exuviae	Weight of honey-dew	TOTAL INGESTED	Rate of ingest-ion	Rate of excret-ion	Rate of assi-milation	% excreted	% assim-ilated	% used for body weight increase	Ratio ingest/ excret.
			mg/aphid/71 hr					mg/aphid/10 hr					
Perfection	2.57	1.47 (0.32)	1.60	0.05	7.53	10.65	1.50	1.06	0.44	71	29	14	1.4
Lincoln	2.38	1.37 (0.06)	1.45	0.05	4.68	7.55	1.06	0.66	0.40	62	38	18	1.6
Ch. of England-1	2.13	1.27 (0.32)	1.12	0.04	3.36	5.79	0.82	0.47	0.35	57	43	22	1.7
Laurier-12	2.49	1.45* (0.12)	1.48	0.05	3.14	6.12	0.86	0.44	0.42	51	49	24	2.0
Melting Sugar	2.29	1.12 (0.22)	1.57	0.04	3.07	5.80	0.82	0.43	0.39	52	48	19	1.9
Daisy	2.04	1.12 (0.46)	1.20	0.04	2.49	4.85	0.68	0.35	0.33	51	48	23	1.9

* Antibiosis was not shown by Laurier-12 probably because of the short duration (71 hr) of this experiment. Decrease in aphid growth rate is more apparent after 4 to 7 days.

** Standard deviations in parentheses.

Champion of England-1. PATHAK & PAINTER (1958) reported that biotypes of the corn leaf aphid, *Rhopalosiphum maidis* (Fitch), gained significantly more weight on susceptible than on resistant sorghum plants, and they concluded that aphids on susceptible plants ingested larger amounts of material. In the present results (Table III), the rates of ingestion and of excretion were higher on the susceptible hosts, and they decreased with increasing host resistance. Relative aphid feeding can therefore be estimated indirectly by a measure of excretion rates. A greater proportion of the material ingested was excreted and a lesser proportion assimilated by the aphids feeding on susceptible than on resistant varieties (Table III). This might indicate that aphids on resistant plants were in a semi-starved condition and utilised more of the lesser amounts of material ingested. This is substantiated by the fact that less amino acids were excreted on resistant plants (Table II). Body evaporation resulted in weight loss about equivalent to measured body weight increase (Table III). For instance in 71 hr, each aphid on Perfection gained about 1.47 mg. in weight and lost 1.60 mg. through evaporation. Aphids also ingested the equivalent of their own weight in food in 12 and 22 hr when feeding on Perfection and Daisy respectively. This gave a much lower ratio of fresh food/fresh insect than those recorded for other aphid species (KENNEDY & STROYAN, 1959) (e.g. 1 to 2 for the pea aphid as against 7.9 to 31.8 for the willow aphid, *Tuberolachnus salignus* (Gmelin), based on a 24-hr food intake).

Aphids on resistant varieties grown under greenhouse conditions therefore feed and excrete at a lower rate, their growth is accordingly reduced and the final body weight attained lower. Some factors (e.g. the turgor pressure and the nutritional value of the plant-host) that may influence the rate of aphid feeding on different pea varieties have been discussed by AUCLAIR (1958b, p. 11—12), and other pertinent information may be found in the review on aphid biology by KENNEDY & STROYAN (1959).

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RÉSUMÉ

L'INGESTION ET L'EXCRÉTION CHEZ LE PUCERON DU POIS, ACYRTHOSIPHON PISUM *(HARR.), ÉLEVÉ SUR DES VARIÉTÉS DE POIS*

La production de miellat par le puceron du pois, *Acyrthosiphon pisum* (Harr.), élevé isolément et en groupes a été mesurée sur des variétés sensibles et résistantes de pois, *Pisum sativum* L., cultivées en serre. La production de miellat par les pucerons isolés fut interrompue au moment de la mue durant les périodes moyennes de 12 et 16 hr; elle fut interrompue entre les mues et aussi au stade adulte durant des périodes moyennes de 3 à 15 hr, même lorsque les aphides en observation paraissaient se nourrir, le bout de leur rostre étant placé sur les nervures des feuilles. Le volume moyen des gouttelettes, la fréquence et le taux d'excrétion furent en général proportionnels au degré de sensibilité de la variété-hôte. Le taux d'ingestion de ces insectes fut plus élevé lorsqu'ils se nourrissaient sur les variétés sensibles, contribuant ainsi à accélérer leur croissance. Une plus grande proportion de la matière ingérée fut excrétée et une proportion moindre assimilée sur les plantes sensibles. Le miellat des pucerons se nourrissant sur les variétés sensibles avait une teneur légèrement plus élevée en amides et acides aminés.

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EXCRETION AND WATERBALANCE IN SOME LAND-ISOPODS

BY

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Land Isopods lose water through evaporation from their body surface, and particularly the respiratory organs, and with excretion and defecation. Water is taken up mainly through drinking and with food.

As regards the structure of respiratory organs, permeability and resistance to high temperatures, there is a sequence in adaptation in three species which have been frequently studied from this point of view, from *Oniscus asellus*, which is least adapted, over *Porcellio scaber* to *Armadillidium vulgare*, which is best adapted to land life.

A technique is described to estimate the water content for small wet objects like the faecal pellets of these species, which lose water rapidly after being produced. The results show that all species, as a rule, extract water from food, but that *Oniscus* does so least, and *Armadillidium* most. Here again, then, is found the same sequence of adaptation, as in the other aspects.

Therefore the adaptation to land life does not concern a number of isolated characteristics of the animal but the organism as a whole.

One of the main problems confronting the animal on land is how to obtain enough oxygen without losing too much water. For respiration a close contact between air and the living tissue is desirable but this entails the danger of losing too much water. Evaporation can be reduced by covering the tissues with a layer which is little permeable to water, but this at the same time reduces respiration. The respiratory surface is sometimes invaginated which reduces water loss to a minimum compatible with the necessary oxygen uptake.

A quite different way of reducing water loss is the choice of habitat. An animal in surroundings with low saturation-deficit will lose water much less rapidly than when occupying a drier locality.

Finally the effect of excessive evaporation from the body surface can be counterbalanced by increased uptake of water.

Adaptation to land life can manifest itself in any one of these aspects of the water balance of animals.

In the land-inhabiting Isopods, the group of Crustaceans which is best adapted to land life, the respiratory organs are the same as those of their nearest water-inhabiting relatives, viz. the pleopods. These are flattened and lie close to the ventral surface of the pleon, the exopodites entirely covering the endopodites. For a detailed description the reader is referred to UNWIN (1932).

The water-inhabiting relatives of the land Isopods perform regular ventilation movements with these pleopods. The pleopods of the land-inhabiting species are generally immobile but may be moved under certain circumstances. When these animals are immersed in water, rhythmical movements are immediately started, but the effect is comparatively slight. No clear current is set up in the water, as in the water-inhabiting species, and the animals can only live for a short time under water.

In some genera, of which *Oniscus* is an example, there are no special structures for respiration on land. In others, such as *Porcellio* and *Armadillidium*, we find invagination in the exopodite of the first and second pair of pleopods, the corpora alba, which are specialized respiratory surfaces. The name pseudotrachaeae, sometimes used, is misleading as the oxygen is passed on to the body fluid, and not directly to the tissues, as the word trachaeae might suggest.

From a morphological study of these last two genera it is clear that *Armadillidium* shows a further increase in adaptation. The corpora alba are relatively larger and the respiratory function is certainly more concentrated in these "new" organs.

It is obvious, therefore, that we can consider the sequence of *Oniscus - Porcellio - Armadillidium* one of progressively better adaptation to land life as regards methods of respiration. This sequence has been repeatedly pointed out by others and has also been correlated with the main habitat of the species of these genera (MEYNERTZ (1943), MILLER (1938), EDNY (1954), and others). *Oniscus* is found in the most humid habitat, *Porcellio* in somewhat drier places, and *Armadillidium* in the driest. It should be remembered, however, that even *Armadillidium* can only live for any length of time in relatively moist surroundings.

Besides these morphological differences, others have been shown to exist. EDNEY (1951) found that evaporation through the cuticle is strongest in *Oniscus asellus,* less so in *Porcellio scaber*, and least in *Armadillidium vulgare.* Closer study of the relation between temperature and evaporation rate showed that evaporation tended to increase gradually as the temperature was raised. There was no sharp change in evaporation rate anywhere between 20° and 60° C, as has been found in insects, indicating that there is no waxlayer covering the cuticle. According to BURSELL (1955) there is such a layer at the inside of the cuticle, but his arguments are not entirely convincing, and anyway such a layer is certainly very much less efficient than in most insects.

EDNEY (1951) further showed that high temperatures can best be sustained by *Armadillidium,* a little less so by *Porcellio*, and least of all by *Oniscus.* The physiological basis for this difference is not clear, but obviously this can be considered in the light of adaptation to land life. On land the risk of extreme high temperatures is much greater than in water.

In the water balance of an animal water can be lost by different routes, the main ones being directly through the body surface, by way of the respiratory surface, but also with excretion and defecation. The study by EDNEY of the loss through the body surface and respiratory surfaces has already been mentioned. For an understanding of the total water balance excretion and defecation must also be considered.

In insects the products of the excretory system are released into the hind gut and are disposed of, together with the faeces. In the Malpighian tubules a certain amount of water is resorbed to produce a more or less dry urine, and in the hind gut of certain species of insects structures may be found which reduce the water content of the faeces before they are expelled through the anus.

In Isopods the faecal pellets are the product of the intestine only, the maxillary glands being generally recognized as the organs for disposing of the waste-products from the blood. We can therefore study the part of the intestine in the water balance of Isopods apart from the excretory system. In this paper we are only concerned with the function of the intestine.

TECHNIQUE

For this study three species of Isopods were used: *Oniscus asellus* L., *Porcellio scaber* Latr. and *Armadillidium vulgare* (Latr.). All three were collected from the Botanical Garden of the University at Leiden. During the experiments they were fed on dead leaves which had been washed beforehand so that no sand or other foreign material adhered to them.

The water content of the food was estimated by weighing a fresh sample of about 1 gm of the leaves, and weighing again after drying over phosphorus-pentoxide until constant weight. Samples were taken in duplicate which mostly differed only slightly, but occasionally gave a difference of 5—6% in water content. This is due to the fact that dead leaves are not homogeneous as regards water content, probably because the larger veins contain less water than the mesophyll.

By allowing water to evaporate the water content of the food was varied between 85% and 55% of the fresh weight.

Direct estimation of the fresh weight of faecal pellets is not possible as they lose water very quickly, owing to their small size. To obtain a reliable value for their weight the following procedure was adopted.

About 25 specimens of one species of Isopod were taken from the glass jar in which they had been feeding for at least 48 hours and put into a large clean petridish, and the dish was covered with a lid. The animals were closely watched and as soon as a faecal pellet was produced a stopwatch was started. The pellet was quickly transferred to the scale of a torsion-balance. This manipulation takes only a few seconds.

The torsion-balance used weights to a maximum of one milligram and can be read to 0.002 mg accurately.

The handle of the torsion-balance was now turned until the torsion was just insufficient for equilibrium with the weight of the pellet. As the water evaporates from the pellet, the weight diminishes and the indicator slowly approaches the equilibrium line. As it passes the line the stopwatch is read. (It is convenient to have a stopwatch with two indicators, running simultaneously; one can be stopped separately at the required moment and read, while the other continues its run).

We thus have the time at which a certain weight was reached. The handle is now again turned to a lower weight, the indicator moves back over the equilibrium line and then again begins approaching, due to water loss of the pellet. The time at which the indicated weight is reached is again read and this can be repeated a number of times.

The points thus found can be plotted in a diagram, the abscissa giving the time, the ordinate the weight. By extrapolating back to time zero the weight of the pellet at the moment of production can be assessed.

A reliable extrapolation is obviously only possible if a straight line can be drawn through the points. From a few examples given in fig. 1 it will be seen that this is the case quite frequently. It may be emphasized that for the actual extrapolation pencil points and lines are drawn, which give a much more accurate result than the ink copy made for this figure. The line of course is always slightly hollow, but for the first few minutes this can be neglected in most cases. Generally 4 points were taken, which mostly took less than 4 minutes. Lateron the bend in the line becomes more evident. After 15—20 minutes there is a further more sharp curvature and the line then becomes nearly horizontal when equilibrium with the surrounding atmosphere is approached.

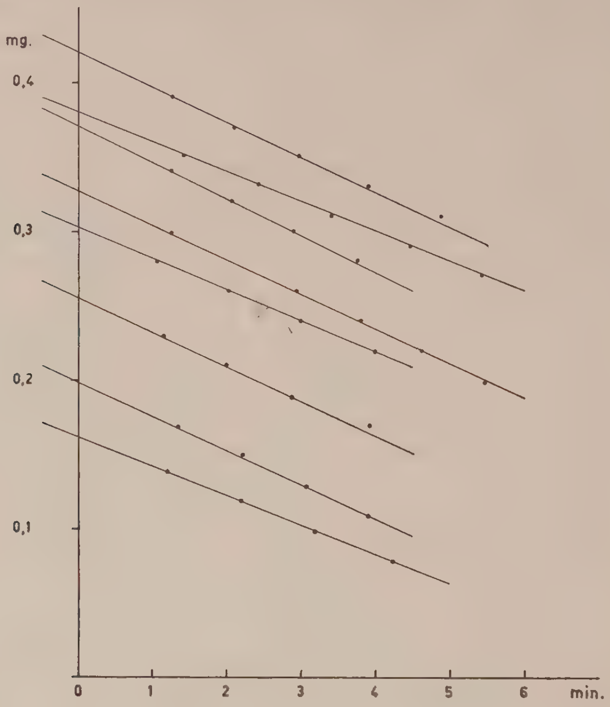

Fig. 1. Extrapolation to estimate initial weight of faecal pellets of Isopods.

Occasionally even the first points did not give a straight line. These results had to be discarded as no proper extrapolation was possible. This occurred only in pellets of very low water content. This possible source of error will be considered further below. After determining the wet weight in the way just described each pellet was put into an exsiccator over P_2O_5 until constant weight.

All fresh weight determinations were made in the morning-hours as the production of faeces is then higher than in the afternoon. This is because feeding is much more intense during the night.

RESULTS

Determinations were made of the water content of the food and of the wet weight and dry weight of the faecal pellets. As there was always superabundance of food, the amount of food can be considered constant.

There was no relation between the size of the faecal pellet and the water content of the food. The diagram, which is not reproduced here, gave a complete random scatter of the points of each species.

The size of the pellet was significantly different in each of the species as is shown in the 3rd vertical column of Table I. *Porcellio* produces small pellets, and those of *Oniscus* are the largest, but this has no further bearing on the problem of water balance.

TABLE I

Faecal pellets of three species of Isopods. n = number of pellets on which the data are based.

	n	mean fresh weight in mg	mean dry weight in mg	percentage water
O. asellus	90	0.546 ± 0.024	0.191 ± 0.020	63.4% ± 1.1
P. scaber	83	0.309 ± 0.014	0.147 ± 0.006	50.1% ± 1.4
A. vulgare	70	0.490 ± 0.023	0.269 ± 0.011	43.0% ± 1.1

More interesting is the fact that the percentage water of the faecal pellets of each of the species is also significantly different as shown in the last column of Table I. *Oniscus* loses most water with defecation, *Porcellio* less and *Armadillidium* produces the dryest faeces.

Now these data give the mean from all determinations with varying water content of the food. A further analysis is possible if we study the variation within the species with the water content of the food. This can be done by considering fig. 2. Here the water content of the food (abscissa) is plotted against the water content of the faecal pellets. The straight line connects the points of equal value for food and pellet.

Each point is the mean of 2—5 determinations, made within one hour, from a group of animals of the same species. All specimens of the three species were kept together in the same container so that the food available to all was the same. Generally the determinations were on all three species one after the other. Occasionally only two or one species was used.

The first point to appear from this figure is that in nearly all cases the faecal pellets are drier than the food. This means that water can be extracted from the food to a smaller or greater degree.

There are four points which lie below the level of equal water content of food and faeces, which means that the faeces were wetter than the food. As it is improbable, though by no means impossible, that water was added to the contents of the intestine during its passage through the body, I believe this result must be due to the fact that the animals can select their food. Under the relatively dry conditions which occurred in this case they must have selected the wettest food obtainable. As has been mentioned before, dead leaves are not homogeneous as

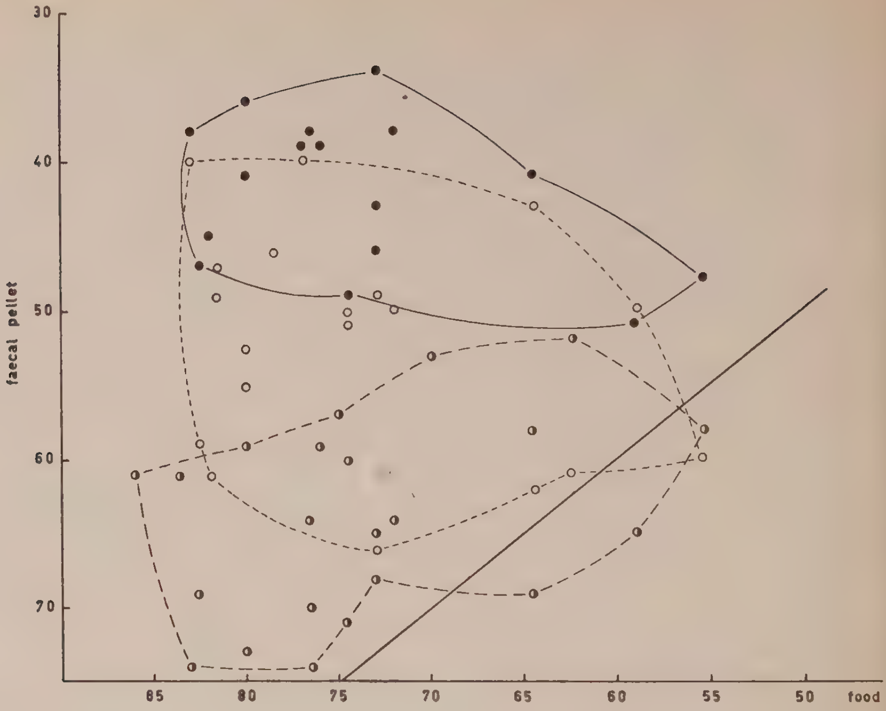

Fig. 2. Relation between water-content of food and faecal pellets in Isopods:

half open circles = *Oniscus asellus*
open circles = *Porcellio scaber*
closed circles = *Armadillidium vulgare*

regards water content, and the veins are relatively drier. This point, however, requires further investigation.

Secondly the difference found between the three species in the overall results (Table I) is again seen in this figure. To make this more obvious the outer values for each species have been connected by a line. This shows the clear distinction between *Armadillidium* and *Oniscus,* with *Porcellio* at an intermediate position.

Further, this figure shows some indication that *Oniscus* tends to extract more water from the food as the food becomes drier, but it needs a lot more data before this can be established with certainty. In the other species no such tendency appears from this figure.

As mentioned before a few dry pellets are left out because the weight could not be determined accurately. Had these values been included it is probable that a few points should have to be moved up a little. But as these undeterminable pellets were nearly all from *Armadillidium*, the difference between the species would then only have been greater.

DISCUSSION

The results described show that generally all species are able to extract water from their food. In this way they can make up at least in part for the water lost by evaporation. *Armadillidium* can extract water from its food best, and *Oniscus* is least capable of doing so.

Drinking of water can be frequently observed in the laboratory in Isopods which have lost considerable amounts of water. (All three species can lose up to 25% of their weight without dying if they are then given opportunity to take up water again quickly).

I have no observations of drinking under natural circumstances, but it cannot be a frequent occurrence. Too much water on the cuticle of the body blocks the pleopods and inhibits respiration, and it is therefore not surprising to find that land Isopods appear to avoid contact with liquid water. They never occur naturally in wet places. Under these circumstances there will be very little occasion for drinking, and water loss can no doubt be counterbalanced by uptake of water from food. But the paper by MELLANBY & FRENCH (1958) on drinking habits of insects should make us careful not to take for granted what has not been studied.

As regards water extraction from food we thus find the same sequence in this respect, as has already shown to exist in other respects of the water balance.

This is not as self evident as might seem. The three species all live on land and though there is some difference in habitat as a group they are poorly adapted to land life. It could very well be imagined that one species is adapted in one way, and others in another way. The greater loss of water through the cuticle of *Oniscus* could be made up for by its greater ability to extract water from its food.

But this does not seem to be the case. As far as our knowledge goes at present we must conclude that in these three species the difference in adaptation runs parallel in all aspects studied. Therefore the adaptation to land life does not concern a number of isolated characteristics of the animals but the organism as a whole.

It should be added that this difference in adaptation explains only one half of the problem of the distribution of the species. It follows from these considerations why *Oniscus* cannot live in the drier habitat, and why *Armadillidium* can. But it does not explain why *Armadillidium* does not occur frequently in the more humid places. As yet there is no evidence of competition in the humid places in favour of *Oniscus* as could be postulated on theoretical grounds.

ZUSAMMENFASSUNG

EXKRETION UND WASSERHAUSHALT BEI EINIGEN LANDISOPODEN

Landtiere sind vor das Problem gestellt, genügend Sauerstoff aufzunehmen, ohne zu viel Wasser zu verlieren. Anpassungen an das Landleben finden sich daher in der Struktur der Körperhülle, im Bau der Respirations- und Exkretionsorgane sowie im Verhalten.

Bei Landisopoden zeigt sich unter den bisher untersuchten Aspekten: der Kutikula-Permeabilität, der Respirationsorgane und der Resistenz gegen hohe Temperatur, eine Reihe, welche von *Oniscus asellus* über *Porcellio scaber* zu *Armadillidium vulgare* von geringerer zu besserer Anpassung fortschreitet.

Im vorliegenden Aufsatz wird die Funktion des Darmes im Wasserhaushalt untersucht. Eine Methode wird beschrieben, mit welcher der Wassergehalt der Faeces bestimmt werden kann. Dazu wird das Gewicht zu verschiedenen Zeitpunkten nach dem Augenblick der

Produktion ermittelt und das Initialgewicht durch graphische Extrapolation nach dem Nullpunkt der Zeitachse festgestellt (Fig. 1.).

Die Trockengewichte von Futter und Faeces werden über P_2O_5 bestimmt.

Es zeigt sich, daß der Wassergehalt der Faeces bei *Oniscus* am höchsten und bei *Armadillidium* am geringsten ist (Tabelle I), während alle Arten dem Futter mehr oder weniger viel Wasser entnehmen können (Fig. 2).

Armadillidium ist also auch in dieser Hinsicht am besten an das Landleben angepasst. Das weist darauf hin, daß wir nicht von der Anpassung einzelner Organe und Funktionen sprechen dürfen, sondern daß das Tier als Ganzes betrachtet werden sollte.

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EFFECTS OF STERILIZED FLAX SEED AND STERILIZED SOIL ON FEEDING BEHAVIOUR AND GROWTH OF LARVAE OF *CTENICERA AERIPENNIS AERIPENNIS* (KBY.) (COLEOPTERA: ELATERIDAE)[1]

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Medium sized (average 43.1 mg) and large (average 110.6 mg) wireworm larvae (*Ctenicera aeripennis aeripennis*) were reared in sterilized or unsterilized soil, with sterilized or unsterilized flax seed supplied as food. Sterilized flax was a better food than unsterilized flax, but unsterilized soil improved the results obtained with unsterilized flax.

Differences in feeding behaviour and growth between larvae of *Ctenicera aeripennis aeripennis* (Kby.) and those of *Ctenicera aeripennis destructor* (Brown) with carrot slices and seeds of wheat, flax, barley, rye, and alfalfa have been discussed in a recent paper (Davis, 1959). The effects of sterilized flax seed and of sterilized soil on larvae of *C. a. destructor* have been reported previously (Davis, 1958). To indicate further biological differences between these subspecies the effects of sterilized flax seed and sterilized soil on larvae of *C. a. aeripennis* are outlined in this report.

PROCEDURE

Medium-sized larvae in this investigation weighed 3.6 to 79.6 mg and averaged 43.1. Large larvae weighed 80.1 to 207.2 mg and averaged 110.6. The same procedure was followed as was used with larvae of *C. a. destructor* investigated under the same conditions (Davis, 1958). The larvae, however, were used immediately after collection from the field and were not subjected to a period of storage at low temperatures. All tests were carried out with ten larvae in each of four replicates, except when sterilized flax seed was provided in unsterilized soil; in this case, two replicates of ten larvae each were used.

After the data had been transformed by the arc sine transformation, they were submitted to analysis of variance. Differences caused by the various combinations were determined at the five percent level.

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[1]) Contribution No. 22, Canada Department of Agriculture Research Station, Saskatoon, Canada.

RESULTS

The data on the average weekly incidence of feeding and molting of medium-sized and large larvae and the coefficients of regression of incidence of feeding on incidence of molting are given in Table I.

TABLE I

Average weekly percentages (angular transformations) of surviving medium-sized and large larvae of C. a. aeripennis *feeding and molting with unsterilized or sterilized flax seed as food in unsterilized and sterilized soil for 30 weeks and coefficients of regression of incidence of feeding on incidence of molting.*

Rearing Conditions	Medium-sized Larvae			Large Larvae		
	Feeding	Molting	Coeff.	Feeding	Molting	Coeff.
Unsterilized flax						
unsterilized soil	34.4	12.9	+0.2556	34.1	12.2	+0.1510
sterilized soil	21.7	13.7	+0.3080	23.5	11.8	+0.6284
Sterilized flax						
unsterilized soil	31.3	11.8	+0.5410	26.2	9.4	+0.7689
sterilized soil	32.3	13.8	+0.5001	27.2	10.7	+0.4365
No food						
unsterilized soil	—	9.9	—	—	8.1	—
sterilized soil	—	9.9	—	—	9.5	—
L.S.D. at 5% level						
sterilized flax,						
unsterilized soil	—	—	—	5.1	2.2	—
others	—	—	—	4.2	1.8	—

The percentages of larvae that fed during the week before, the week after, and the week in which molting occurred were as follows:

Time of feeding	Week before molting	Week of molting	Week after molting
Medium-sized larvae			
unsterilized soil			
unsterilized flax	18	4	36
sterilized flax	16	8	43
sterilized soil			
unsterilized flax	12	3	38
sterilized flax	22	6	45
Large larvae			
unsterilized soil			
unsterilized flax	27	0	60
sterilized flax	19	0	46
sterilized soil			
unsterilized flax	3	0	46
sterilized flax	11	3	37

The average number of weeks between molts was as follows:

Type of soil	Unsterilized	Sterilized
Medium-sized larvae		
unsterilized flax	6	5
sterilized flax	7	8
without food	3	4
Large larvae		
unsterilized flax	8	8
sterilized flax	9	7
without food	8	7

Medium-sized larvae gained weight in both types of soil when food was added and lost weight when no food was provided (Table II). Large larvae gained most weight with unsterilized and sterilized flax seed in unsterilized soil.

TABLE II

Average changes in weight in milligrams per milligram of initial weight and percentages (angular transformations) of medium-sized and large larvae of C. a. aeripennis *surviving on unsterilized and on sterilized flax seed in unsterilized and sterilized soil for 30 weeks*

Rearing Conditions	Medium-sized Larvae		Large Larvae	
	Change in Wt.	Survival	Change in Wt.	Survival
Unsterilized flax				
unsterilized soil	1.20	47.9	0.28	37.6
sterilized soil	0.55	24.5	-0.01	56.1
Sterilized flax				
unsterilized soil	0.88	77.1	0.17	83.3
sterilized soil	1.28	76.2	0.13	68.9
No food				
unsterilized soil	-0.22	36.7	0.01	85.4
sterilized soil	-0.27	33.8	-0.07	65.8
L.S.D. at 5% level				
sterilized flax, unsterilized soil	1.07	31.5	0.14	28.8
others	0.87	25.7	0.12	23.5

A greater percentage of medium-sized larvae survived with sterilized flax than with unsterilized flax or without food (Table II). As many large larvae survived with sterilized flax in both soils and with unsterilized flax in sterilized soil as without food. Fewer survived when neither flax nor soil was sterilized than in any other instance.

DISCUSSION

Medium-sized larvae of *C. a. destructor* fed more frequently on unsterilized than on sterilized flax seed, gained more weight with the latter, and survived best with unsterilized flax seed in sterilized soil (DAVIS, 1958). In contrast, medium-sized larvae of *C. a. aeripennis* were attracted as much to sterilized as to unsterilized flax seed and gained as much weight on the one as on the other. Also, more medium-sized larvae of *C. a. aeripennis* survived with sterilized than with unsterilized flax seed. The data on survival suggest that sterilization may have eliminated some factor or factors in flax seed, toxic to medium-sized larvae of *C. a. aeripennis,* as it did with corn germ fed to larvae of *Tenebrio molitor* L. (LIPKE & FRAENKEL, 1955).

Large larvae of *C. a. destructor* fed most frequently on unsterilized flax in unsterilized soil. They gained more weight and survived better with sterilized flax in sterilized soil than in any other case (DAVIS, 1958). Large larvae of *C. a. aeripennis* differed from them only by gaining most weight with unsterilized or with sterilized flax seed in unsterilized soil. Apparently, although more large larvae of *C. a. aeripennis* survived with sterilized than with unsterilized flax in unsterilized soil, sterilizing the seed did not affect them because survival was no greater in this case than when unsterilized or sterilized flax was provided in sterilized soil, or when no flax was supplied.

Data obtained without flax both with larvae of *C. a. destructor* (DAVIS, 1958) and with larvae of *C. a. aeripennis* (Table I, II) indicated that micro-organisms in the soil were probably not fed on by wireworms of these species. They suggested, in the case of large larvae of *C. a. aeripennis*, that soil micro-organisms may be capable of making some factor or factors in the flax seed more readily available to the larvae, thus permitting the larvae to gain more weight with sterilized or with unsterilized flax in unsterilized soil.

SOMMAIRE

EFFETS DE LA SEMENCE DE LIN ET DU SOL STERILISÉS SUR LE COMPORTEMENT DE NUTRITION ET LA CROISSANCE DES LARVES DU TAUPIN CTENICERA AERIPENNIS AERIPENNIS *(Kby.)*

Dans un effort de détérminer les différences biologiques entre le taupin des graminées, *Ctenicera aeripennis destructor* (Brown), et celui de Puget Sound, *Ctenicera aeripennis aeripennis* (Kby), les effets sur les larves de ce dernier de la semence de lin et du sol sterilisés fut observés pendant 30 semaines. Contraire aux résultats obtenus avec les larves de *C. a. destructor*, le lin sterilisé autant que le lin non-sterilisé prouva attrayant et efficace pour la croissance aux larves de taille moyenne de *C. a. aeripennis*. Les larves de *C. a. aeripennis* de grosse taille ne différèrent de celles de *C. a. destructor* que par une plus grande augmentation de poids quand le lin sterilisé ou non-sterilisé fut présent en sol non-sterilisé. La survivance des larves de grandeur moyenne fut meilleure avec le lin sterilisé qu'avec le lin non-sterilisé tandis que celle des grosses larves ne fut pas influencée.

Les résultats obtenus sans le lin avec les larves des deux espèces suggèrent que les microorganismes dans le sol ne sont probablement pas utilisés dans l'alimentation de ces espèces. Dans le cas des larves de *C. a. aeripennis* de grosse taille, il est fort probable que les microorganismes dans le sol rendent plus accessibles aux larves des facteurs alimentaires du lin, ce qui leur permet une croissance meilleure que d'ordinaire.

Pourvues du lin, 25 p.c. des larves de grandeur moyenne mangèrent hebdomadairement. A peu près de 5 p.c. muèrent chaque semaine, même sans lin. Avec le lin non-sterilisé, 17 p.c. des larves survécurent dans le sol sterilisé, tandis que 55 p.c. survécurent en sol non-sterilisé. Avec le lin sterilisé, plus de 90 p.c. survécurent, et dans le sol non-sterilisé et dans le sol sterilisé. Pourvues du lin, les larves prirent du poids; sans lin, elles en perdirent.

Avec le lin sterilisé dans le sol sterilisé, 31 p.c. des larves de grosse taille mangèrent chaque semaine et seulement 16 p.c. dans tout autre cas. A peu prés 4 p.c. muèrent chaque semaine. Seulement 37 p.c. des larves de cette taille survécurent dans le sol non-sterilisé

avec le lin non-sterilisé, mais plus de 98 p.c. survécurent avec du lin sterilisé dans le même sol et plus de 65 p.c. dans le sol sterilisé pourvu de lin ou sans lin.

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EFFECTS OF DRY AND GERMINATING WHEAT ON FEEDING BEHAVIOUR AND GROWTH OF LARVAE OF THE PRAIRIE GRAIN WIREWORM, *CTENICERA AERIPENNIS DESTRUCTOR* (BROWN) (COLEOPTERA: ELATERIDAE)[1]

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If larvae start to feed on dry or germinating wheat, they take as much food, and a similar proportion of embryo and endosperm, at each feeding. However, they feed twice as frequently on germinating wheat than on dry wheat, and therefore consume twice as much of the former than of the latter. It is suggested that an olfactory attractant may diffuse out from germinating wheat. The larvae gained as much weight on embryo or endosperm as on whole kernels of germinating wheat. The gain in weight on each of these foods was significantly greater than on hard seeds. Forty-four percent of those provided with whole dry seeds, 22 percent with whole germinating seeds, 26 percent with endosperm and 18 percent with embryo of germinating wheat moulted during the 28-day period. No diapause occurred in larvae fed only on endosperm of germinating wheat for 80 days.

When larvae of *Ctenicera aeripennis destructor* (Brown) were reared in soil on seeds of wheat, flax, barley, rye, and alfalfa (Davis, 1957), no information on the incidence of feeding was obtained because only weekly observations were made. Because whole seeds were provided the effect of embryo or endosperm on the larvae was not shown. The effects of whole, dry wheat and whole seed, embryo, and endosperm of germinating wheat on feeding behaviour and growth of larvae of *C. a. destructor* are reported here.

MATERIALS AND METHODS

Larvae that had been stored for five months in moist soil at 30° C. were weighed to the nearest 0.1 mg before use. The initial weights ranged from 4.2 to 13.4 mg and averaged 7.7. Fifty test tubes, prepared as for aseptic rearing (Davis, 1959) but without pellets of synthetic diet, were used for each food tested. One dry wheat seed, one germinating wheat seed, one wheat embryo, or one wheat endosperm was substituted for the pellet of diet in each test tube. Germinating seeds were obtained by placing dry seed between layers of moist filter paper in a Petri dish at 30 ± 2° C. and 60 ± 5 percent relative humidity for 24 hours. To provide wheat embryo, the germ was excised from the germinating seed with dissecting forceps.

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[1] Contribution No. 18, Canada Department of Agriculture Research Station, Saskatoon, Sask.

After one larva had been added to each, the test tubes were stoppered with absorbent cotton and were placed in a constant temperature cabinet at 18 ± 2° C. and 50 ± 5 percent relative humidity. They were checked daily for 28 days; feeding, moulting, and survival were recorded; and the food in the tubes was changed. After 28 days, the larvae were removed from the tubes and were weighed to the nearest 0.1 mg. Ten larvae that had fed on endosperm and had moulted were kept on this food for 52 days longer to determine whether diapause would occur.

To estimate the amount eaten daily by each larva, scores from 1 to 5 were given, both for embryo and for endosperm; 1 indicated only a nibble on the food; 2, one-quarter of the food eaten; 3, one-half of the food; 4, three-quarters of the food; and 5, all the food.

Data were analysed by analysis of variance.

RESULTS AND DISCUSSION

Table I summarizes the data on feeding incidence, food consumption, weight gains and survival during the 28-day test period.

TABLE I

The feeding activity, change in weight (milligrams per milligram initial weight), and percentage survival of medium-sized larvae of C. a. destructor *reared for 28 days in test tubes containing moist absorbent cotton substrate with dry wheat and whole seed, embryo and endosperm of germinating wheat*

Diet	Average Days Feeding/Larva	Average Proportion Eaten/Larva/Incident of Feeding[1] Embryo	Endosperm & Pericarp	Gain in Weight	Percentage Survival
Hard wheat					
whole seed	4.1	4.1	1.0	0.51	98
Germinating wheat					
whole seed	8.2	3.9	1.1	1.03	94
endosperm	10.9	—	2.3	1.08	94
embryo	15.2	4.6	—	0.90	98
L.S.D. at 5% level	2.6	—	—	0.25	—

[1]) If 1 = nibble on food; 2 = one-quarter eaten; 3 = one-half eaten; 4 = three-quarters eaten; 5 = all eaten.

The endosperm is 87 to 89 percent and the embryo 2.8 to 3.5 percent of the weight of the wheat seed (PERCIVAL, 1921). Thus the larvae provided with only endosperm received smaller food portions, and those with only embryo much smaller portions, than those with whole kernels. Because of this, the method of scoring provided comparable estimates of food consumption only in the case of the whole kernels of dry and germinating wheat. This difference in size of portion among the four foods was also probably a factor affecting the incidence of feeding. Therefore, the incidence of feeding and food consumption are considered only for the whole kernels of dry and of germinating wheat, where the food portions were of the same size. The method of scoring provided more comparable estimates of the consumption of dry and germinating kernels than would have been obtained from the weights of the foods consumed because it compensated for the difference in moisture content.

The larvae fed on twice as many days on germinating as on dry seeds (Table I). Eighty-six percent of the larvae provided with germinating seeds, as compared with only 70 percent of those with dry seeds, fed during the 28-day test period. However the average consumption per day, and the ratio of embryo to endosperm consumed was similar with the two foods.

The feeding behaviour with the two foods was also similar. The larvae nearly always entered the kernels of both dry and germinating wheat through the germ end, possibly because the portion of the pericarp covering the embryo is thinner than that over the remainder of the seed (PERCIVAL, 1921). The percentages of instances when the larvae consumed various portions of the embryo were :

proportion eaten	merely nibbled	one-quarter	one-half	three-quarters	all
dry seed	5.5	5.6	3.8	6.0	79
germinating seed	5.5	11.2	4.7	3.6	75

The fact that the incidence of feeding on the germinating wheat was about double that on the dry wheat, whereas the feeding behaviour and the amount consumed at each incident of feeding was similar with the two foods, suggests that an olfactory attractant may be diffused during germination, and that this may be an important factor governing wireworm attack on wheat seeds.

If the greater water content of the germinating wheat or the chemical changes accompanying germination had appreciably increased the palatability of the seed, one would expect greater consumption of germinating than of dry wheat at each incident of feeding. Thus, the larvae might be expected to attack fewer germinating than dry kernels to satisfy their food requirements. Also, if the hardness of the seed coat were an important factor one would expect the larvae to nibble on a smaller proportion of the germinating than of the dry seed before finding enough penetrable kernels to meet their food requirements.

Whole kernels of dry and germinating wheat are apparently about equal in nutritional value on the basis of the weights that the larvae gained therewith. The larvae ate about twice as much of, and gained about twice as much weight with germinating as with dry seed (Table I). The weights gained with embryo, endosperm and whole kernels of germinating wheat did not differ significantly, and the gain with each was significantly greater than that with dry seed.

When the relative sizes and the proportions consumed of embryo and endosperm are considered in relation to the weight gains with these two foods, it is obvious that the larvae required a much greater quantity of endosperm than of embryo to gain an equal amount of weight.

Forty-four percent of the larvae provided with dry wheat, 22 percent with germinating wheat, 26 percent with endosperm and 18 percent with embryo of germinating wheat moulted during the test period. None moulted more than once.

KOSMACHEVSKII (1950) reported that a diapause occurred in larvae of *Agriotes meticulosus* Cand. that fed on the endosperm of swollen wheat grains. No diapause occurred in the larvae of *C. a. destructor* that were fed daily on endosperms of kernels that were swollen after having been kept between layers of moist blotting paper for 24 hours at 30 ± 2° C. Forty of the larvae were fed on these endosperms for 28 days; ten were fed on them for 80 days [1]).

SOMMAIRE

EFFETS DE LA GRAINE SÈCHE DE BLÉ ET DE LA GRAINE DE BLÉ GERMÉ SUR LE COMPORTEMENT DE NUTRITION ET LA CROISSANCE DES LARVES DU TAUPIN CTENICERA AERIPENNIS DESTRUCTOR *(BROWN)*

Les larves du taupin des graminées, *Ctenicera aeripennis destructor* (Brown), furent élevées individuellement pendant 28 jours dans des éprouvettes pourvues de ouate humide, d'une graine sèche de blé, ou d'un embryon, d'une graine entière de blé germé, et bouchées d'un tampon d'ouate non-absorbante. Les larves mangèrent pendant deux fois plus de jours les graines germées que les graines sèches. Cependant, la consommation journalière et le rapport d'embryon à l'endosperme furent semblables avec les deux graines. Les larves entrèrent presque toujours dans les graines par l'embryon, probablement à cause d'un péricarpe plus mince dans cette région.

Le comportement général des larves suggère qu'un facteur olfactif attrayant soit diffusé pendant la germination et que ce facteur soit du premier rang comme déterminant d'attaque par le taupin. Ni la teneur en eau de la graine, ni la dureté de l'épisperme parut être important à cet égard.

Le poids des larves augmenta également avec l'embryon, l'endosperme, et la graine entière de blé germé et significativement plus qu'avec la graine entière de blé sec. Aucune evidence d'une diapause ne fut observée quand les larves ne mangèrent que de l'endosperme du blé germé pendant 80 jours.

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[1]) The editors would like to draw attention to the publications by KLINGER (1957—59), Über die Bedeutung des Kohlendioxyds für die Orientierung der Larven von *Otiorrhynchus sulcata* F., *Melolontha* and *Agriotes* (Col.) im Boden: *Mitt. Schweiz. Entomol. Gesell.* 30 : 317—322; 31 : 205—269; 32 : 311—316) which also contain data of importance on the orientation of Elateridae in the soil (Ed. Note).

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GENETICS OF RESISTANCE TO ORGANOPHOSPHORUS COMPOUNDS AND LOW ALI-ESTERASE ACTIVITY IN THE HOUSEFLY

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The relation between low ali-esterase activity and organophosphate resistance was studied in a malathion and a diazinon resistant strain of houseflies by a system of repeated back-crosses with a susceptible strain. In the malathion resistant strain G, low ali-esterase activity and resistance are dependent on one and the same autosomal gene. In the diazinon resistant strain F one gene is responsible for the low esterase activity and part of the resistance, whereas at least one other resistance factor is present not affecting the esterase activity. Two subcolonies were obtained, one which is called F_a with only the gene for low ali-esterase activity, the other, called F_b, without this gene but with another resistance factor. Since NGUY and BUSVINE found a single gene to be responsible for resistance in three other strains, a total of five strains has now been found to have an important gene for low esterase activity and resistance to phosphate compounds. These genes, which differ in the degree or specificity of the resistance they confer to the strains, are probably all alleles. It is concluded that physiologically closely related defence mechanisms must be responsible for these different kinds of resistance.

In a previous paper (VAN ASPEREN & OPPENOORTH, 1959) it was shown that five strains of houseflies resistant to organophosphates had only about one fifth of the ali-esterase activity found in six phosphate susceptible strains. It was concluded that the genes for low ali-esterase activity must be responsible for at least part of the resistance of the strains. OPPENOORTH (1959) studied the cross-resistance patterns of the same phosphorus resistant strains. Only a low resistance to diazinon was found in the malathion resistant strain, and only a moderate resistance to malathion in the diazinon resistant strain. If it is assumed that the genes for low ali-esterase activity are the same in all resistant strains, it follows that these genes can be responsible only for the low resistance which the strains have in common. However, it seems unlikely that genes with such a slight influence on resistance would have been selected out in all resistant strains studied. Moreover, the assumption implies a multi-gene basis of the resistance whereas it was found by NGUY & BUSVINE (1960) that in one malathion and in two parathion resistant strains resistance depends on one major gene pair. If the resistance indeed depends completely on one gene, this must be the same which is responsible for low ali-esterase activity. The different resistance patterns in the strains all having an important low esterase factor in common could then only be explained by assuming the presence of different factors for low ali-esterase activity.

To study this intriguing assumption it was attempted to obtain an answer to the following questions:

1. Does the low ali-esterase activity in each strain depend on one or more genes ?
2. Does resistance to organophosphates depend on one or more genes ?
3. What is the relation between resistance factors and factors for low esterase activity ?
4. What is the relation between the genes for low esterase activity present in the different strains ? Could they be alleles or independent genes ?

MATERIAL AND METHODS

Strains

The following strains of *Musca domestica* L. were used:

S, susceptible, with high ali-esterase activity.

F, diazinon resistant, with low ali-esterase activity, originating from Denmark.

G, malathion resistant, with low ali-esterase activity, originating from Georgia, U.S.A. (from the Bethesda 45 colony, FAY, KILPATRICK & MORRIS, 1958).

Strains F and G were chosen since they differed widely in resistance patterns, and had the highest resistance of the colonies present in the laboratory. Moreover strain F had been used in physiological investigations. The resistance patterns of the strains have been previously described (OPPENOORTH, 1959). The flies were reared on a milkpowder-yeast diet at 25° C and ca. 65% relative humidity.

Crossing experiments

The crosses made are shown in Table I. The notation of the crosses and the progenies is described in the legend of this table. A detailed description will be given under "results". The essential part consists of repeated back crossing with strain S followed by selection, either for low ali-esterase activity or resistance. Repeated back crossing has been recommended by WRIGHT (1952) and cited by CROW (1957) as a means for studying the presence of important genes and for isolating them. If a certain character is dependent on one gene it will be present in 50% of the backcross with the susceptible strain, and it should be possible to maintain the character by selection after the backcrosses. In the present study the selection was for low ali-esterase activity, and it was hoped by repeated backcrosses to remove possible other resistance factors, in order to estimate the part of the total resistance that depends only on the genes for low ali-esterase activity.

In studying the relation between low ali-esterase activity and resistance a difficulty is presented by the fact that for the estimation of both properties the flies have to be killed. Therefore, instead of investigating the properties of the flies themselves, their offspring were studied, being partly used in the esterase determinations and partly for the susceptibility measurements. Selection for low ali-esterase activity was possible in two ways. The first possibility is to check the presence of the desired property in the progenies and to breed flies only from progenies in which it is present. The second possibility is to collect egg batches derived from single pairs and to rear them only if the parent flies showed the property wanted.

TABLE I

Scheme of the crosses carried out.

B_1, B_2 = first, second backcross generation, if not indicated otherwise, backcross with S. I_1, I_2 = first, second generation of inbreeding to obtain a homozygous a a *strain. The single pairs and their progenies have been numbered. E.g. if 5 male flies of the progeny of single pair no 3 of the first backcross generation are crossed with S so that five progenies are obtained of this second backcross generation, this is written as S × B_1 3 → B_2 1—5.*

	S × F	
no of single pairs used	cross	name of the progenies of the single pairs
	♀ ♂	
mass cross	$S \times F \rightarrow$	f_1
1	$f_1 \times f_1 \rightarrow$	f_2
5	$S \times f_1 \rightarrow$	B_1 1—5
5	$f_1 \times S \rightarrow$	B_1 6—10
1	$F \times f_1 \rightarrow$	B_1 (F)
6	$S \times B_1\ 3 \rightarrow$	B_2 1—6
6	$B_1\ 3 \times S \rightarrow$	B_2 7—12
10	$S \times B_2\ 8 \rightarrow$	B_3 1—10
5	$S \times B_2\ 11 \rightarrow$	B_3 11—15
5	$S \times B_3\ 2 \rightarrow$	B_4 1—5
8	$S \times B_3\ 6 \rightarrow$	B_4 6—13
8	$B_4\ 6 \times B_4\ 6 \rightarrow$	I_1 1—8
	B_4 5 selected with diazinon →	F_b
8	$I_1\ 8 \times I_1\ 8 \rightarrow$	I_2 1—8. I_2 1, inbred → F_a

	S × G	
no of single pairs used	cross	name of the progenies of the single pairs
	♀ ♂	
mass cross	$S \times G \rightarrow$	f_1
	$f_1 \times f_1 \rightarrow$	f_2
5	$S \times f_1 \rightarrow$	B_1 1—5
1	$G \times f_1 \rightarrow$	B_1 (G)
5	$S \times B_1\ 3 \rightarrow$	B_2 1—5
7	$B_2\ 4 \times B_2\ 4 \rightarrow$	I_1 1—7
8	$I_1\ 7 \times I_1\ 7 \rightarrow$	I_2 1—8
8	$I_2\ 6 \times I_2\ 6 \rightarrow$	I_3 1—8

Techniques of the crossing experiments

Since it was not known whether the strains used were homogeneous and homozygous for genes affecting resistance or esterase activity, in most experiments single pair crosses were used instead of mass crosses. It was hoped that in this way possible segregations would show up more clearly. Virgin females were obtained by isolation of individual pupae, or by removing flies within six hours after emergence from a mass culture. The latter procedure is more convenient if large numbers are required. Single pairs were kept in 3 l bottles, provided with water and food (a mixture of sugar, milkpowder and yeast powder). From the second day after emergence a small cap containing tissue paper moistened with milk was introduced overnight for oviposition. The eggs were brought into small jars with 80 ml of the milk powder-yeast diet to which some nipagine was added as a fungicide, and covered with saw dust. After the larvae had pupated they were counted and transferred into jars with water and food, where they emerged. Generally 2—4 batches of eggs were obtained from one female. Flies were used one or two days after most flies had emerged, when practically all were from one to three days old. The pupae from the last batch of eggs laid by a fly were kept at 9° C, which considerably retarded emergence. In this way virgin females for crossing experiments were available after the results of the tests with flies from the older batches had been obtained.

Ali-esterase measurements

The difference between the ali-esterase activity of susceptible and resistant strains is largest for the substrates ethylbutyrate and methylbutyrate. Since methylbutyrate has the advantage that it is more soluble in water, this substance was chosen here. Measurement of the activity in homogenates of individual flies was desirable, and therefore the method followed by VAN ASPEREN & OPPENOORTH (1959), who used mass homogenates, has been modified as follows.

One fly was homogenized for 30 seconds in an ice cold mixture of 2.3 ml of 1% NaCl solution and 1 ml of an inactivated homogenate in 1% NaCl. The homogenate was quantitatively introduced into the main vessel of a Warburg flask, and 0.3 ml of 1.26% $NaHCO_3$ and 0.4 ml of an emulsion of methyl butyrate in 1.26% $NaHCO_3$ were pipetted into the sidearm.

The emulsion was prepared by mixing 1 ml. of methylbutyrate in 9 ml of 1.26% bicarbonate solution and 0.1 ml of an emulsifier (Atlox 1045 A). The inactivated homogenate was made by heating a homogenate of 1 male fly of strain S per ml of 1% NaCl solution for 5 minutes to 70° C. It was added since in some experiments the greater dilution needed in the work with individual flies seemed to result in too low values as compared with the mass homogenates. This seemed to be counteracted by the addition of the heated homogenate. The effect was not fully analysed, however, and the heated homogenate may not have been necessary. The vessels were kept in ice water until they were attached to the manometers. A gas mixture of 5% CO_2 and 95% N_2 was applied for 5 minutes, the contents of the side arm was tipped in, and after 5 min. of temperature equilibration the readings were taken at 10 min. intervals for 40 minutes.

It should be noted that the data obtained in this way are not directly comparable with those obtained in the mass homogenates, since the conditions, especially the substrate concentration, are different.

Susceptibility measurements

Diazinon susceptibility was measured with the contact method previously described (OPPENOORTH, 1959). Malathion susceptibility was measured with the topical application method described by BUSVINE (1959); 0.5 μl of a malathion solution in olive oil was applied to the dorsal thorax by means of an Agla micrometer syringe.

RESULTS

Ali-esterase activity

In the right parts of figures 1—6 the ali-esterase activities of individual male flies, expressed as μl of CO_2 formed in 30 minutes in the Warburg are marked on a linear scale. Fig. 1 shows the activities of strain S and F and their f_1, f_2 and first backcross generation (B_1). High activity is found in S, low in F and intermediate in f_1. The activities of f_2 flies and of flies obtained in the backcrosses with both parent strains show clear segregation, and lines have been drawn

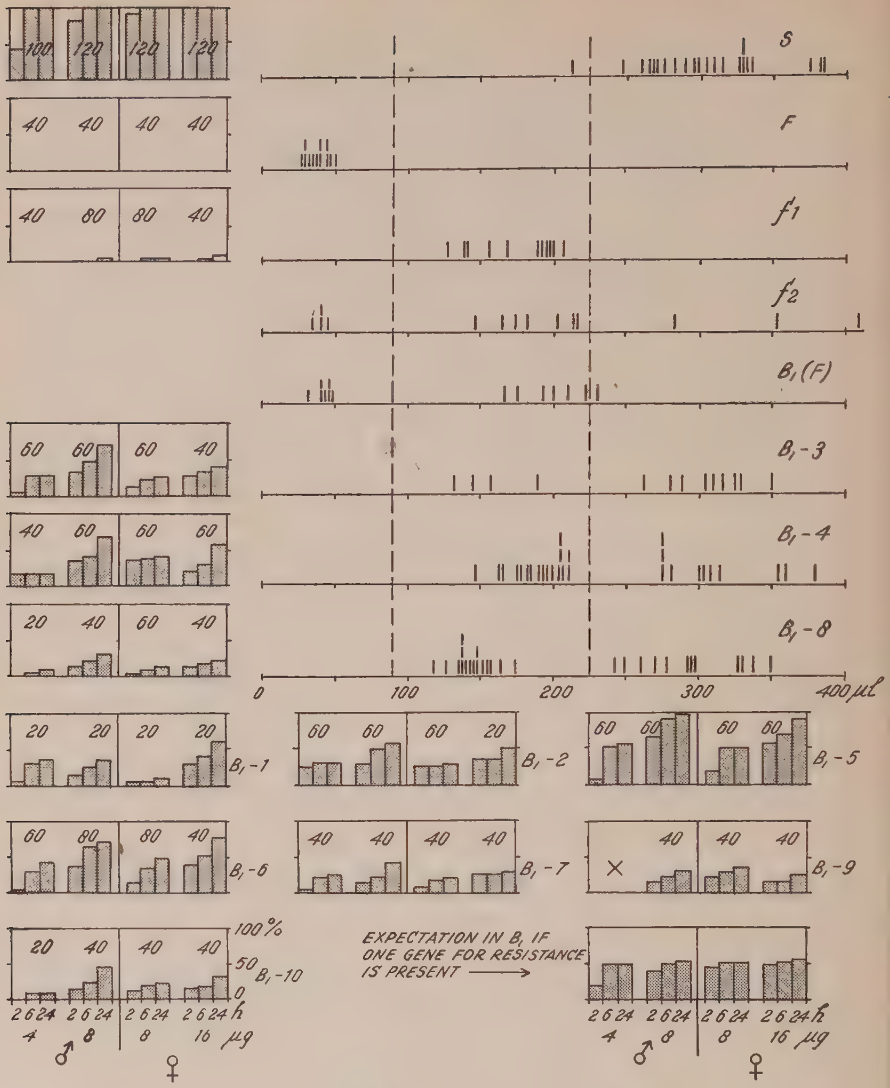

Fig. 1. S × F, f$_1$, f$_2$ and first backcross.

Fig. 1—6. Susceptibility and ali-esterase activity of strains S, F and G and their crossing products.

Ali-esterase activities of individual male flies are represented by dashes, indicating the amount of CO$_2$ in µl produced in 30 minutes. In the backcrosses every line represents the offspring of a single pair. If the sign ♂ or ♀ is present, these indicate the activity found in the parent flies of the progeny plotted on the same line.

In the bar-graphs the percentage of flies knocked down after 2, 6 and 24 hours is given at the concentrations indicated. The figures in the blocks indicate the number of flies used with that concentration. Where a bar-graph is given on the same line as an ali-esterase graph, flies from the same progeny have been used for both estimations.

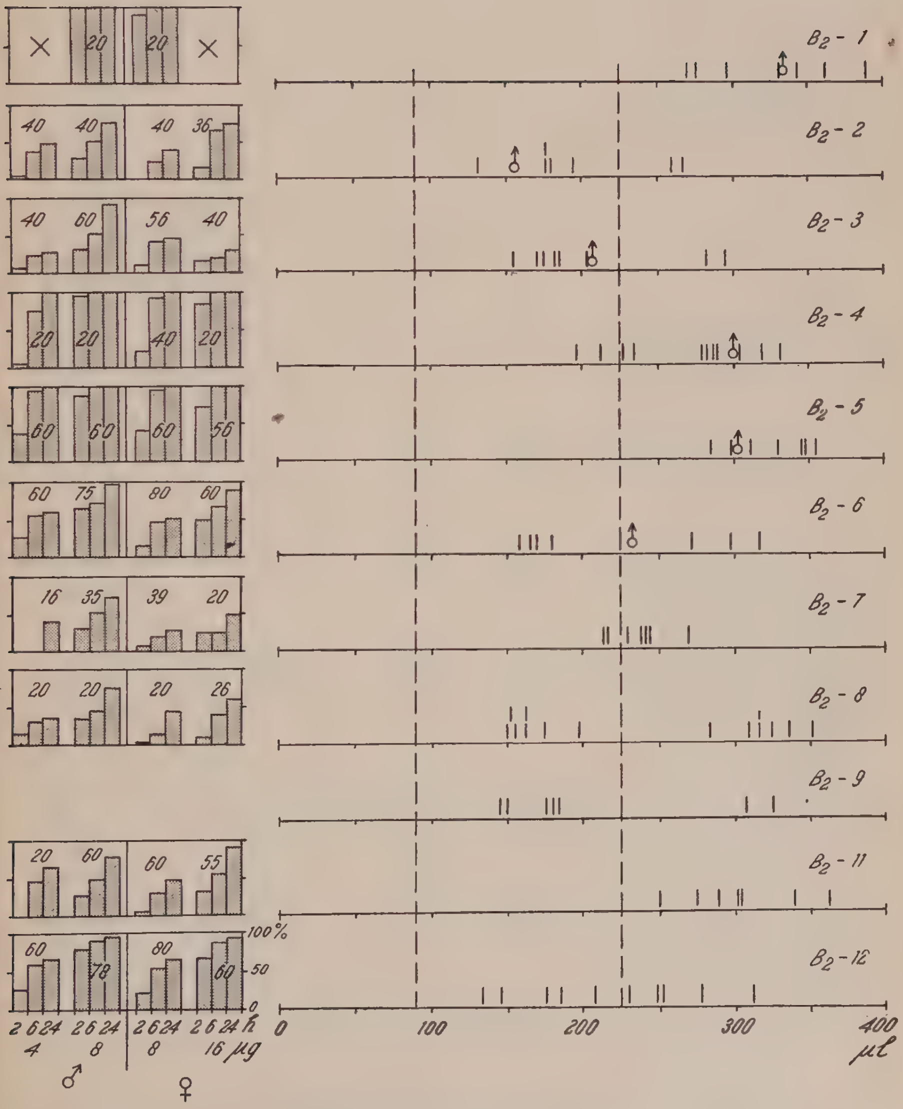

Fig. 2. S × F, second backcross.

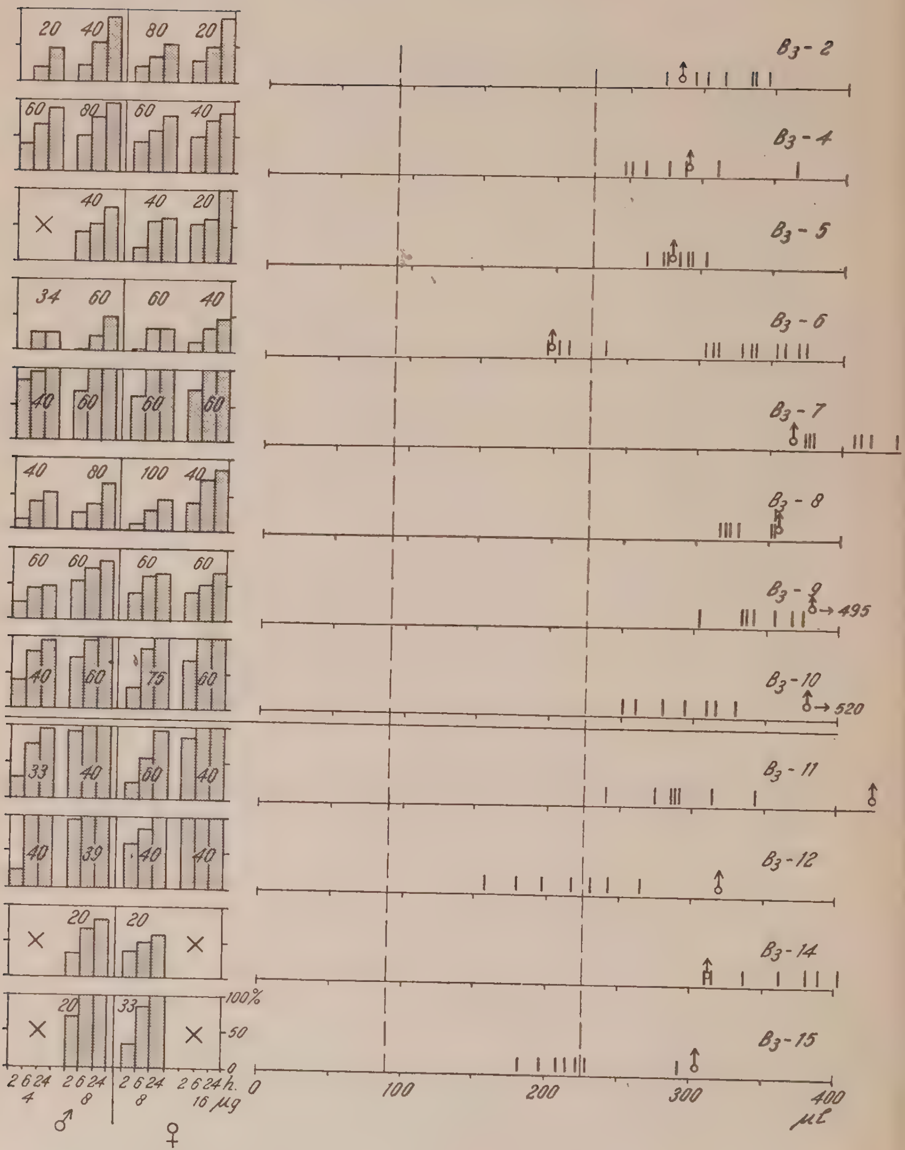

Fig. 3. S × F, third backcross, B3 2—10 from progeny B2 8 with the aa+ group. B3 11—15 from progeny B2 11, without the aa+ group.

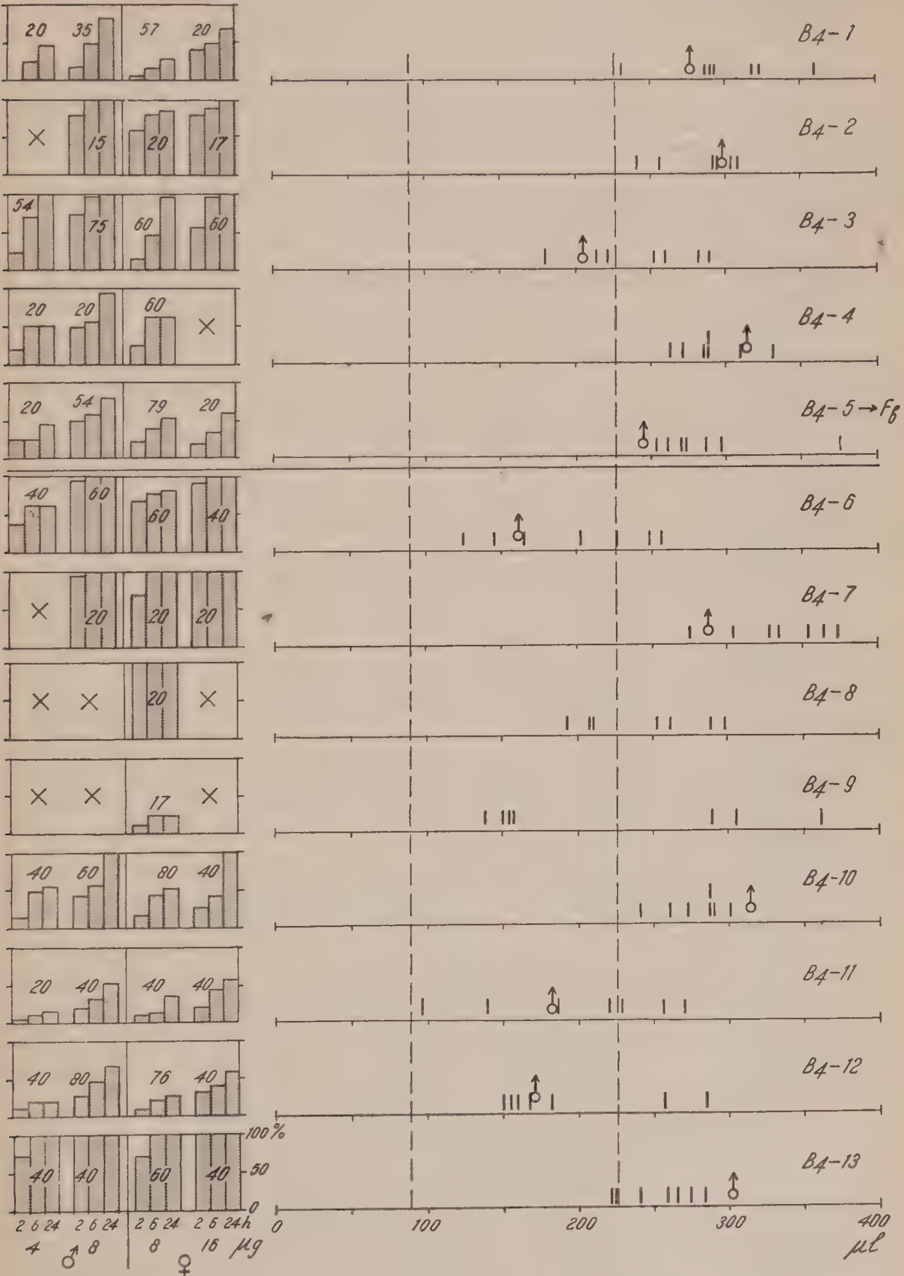

Fig. 1. S × F, fourth backcross, B_4 6—13 from progeny B_3 6 with the aa^+ group. B_4 1—5 from progeny B_3 2 without the aa^+ group.

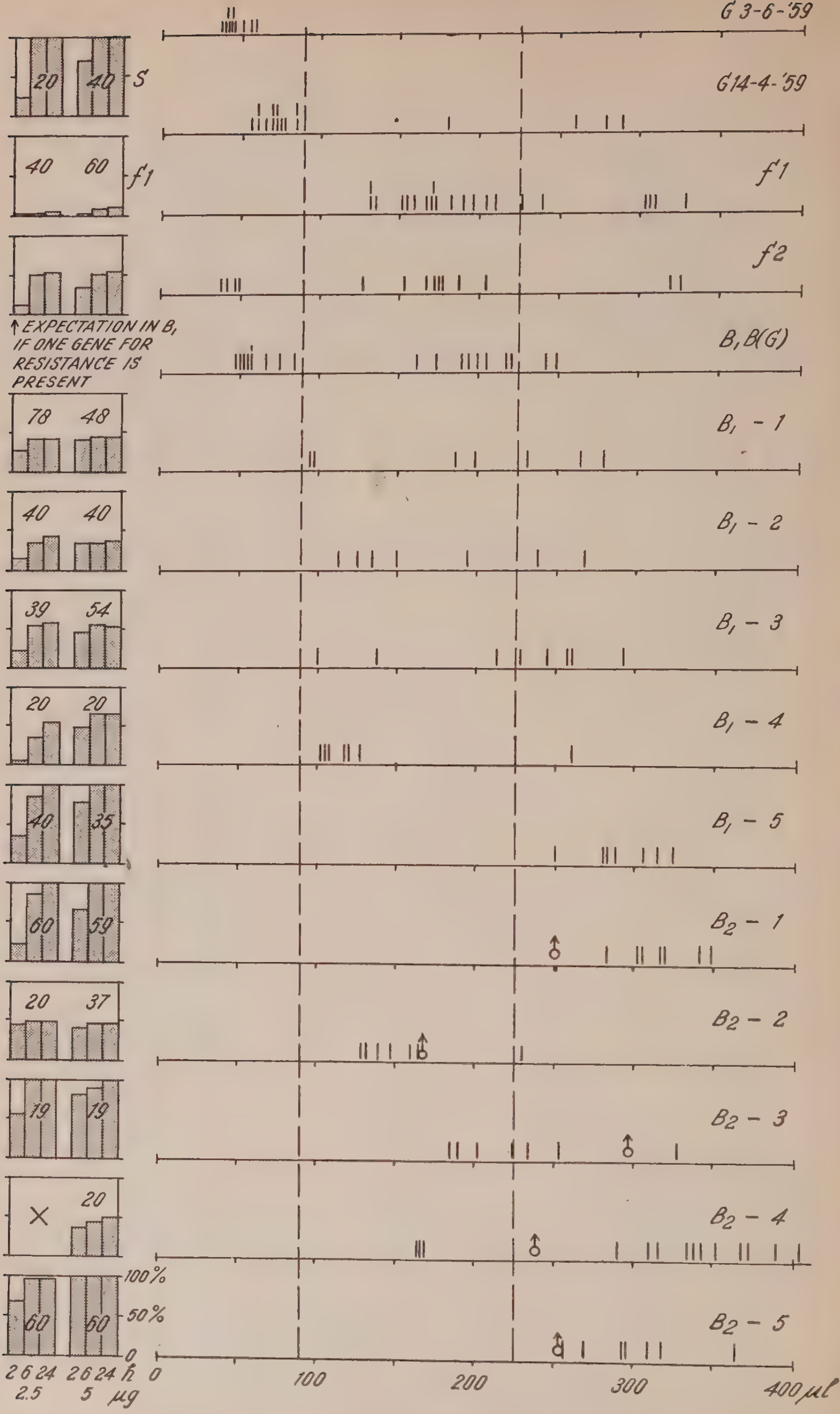

Fig. 5. S × G, f1, f2, first and second backcross.

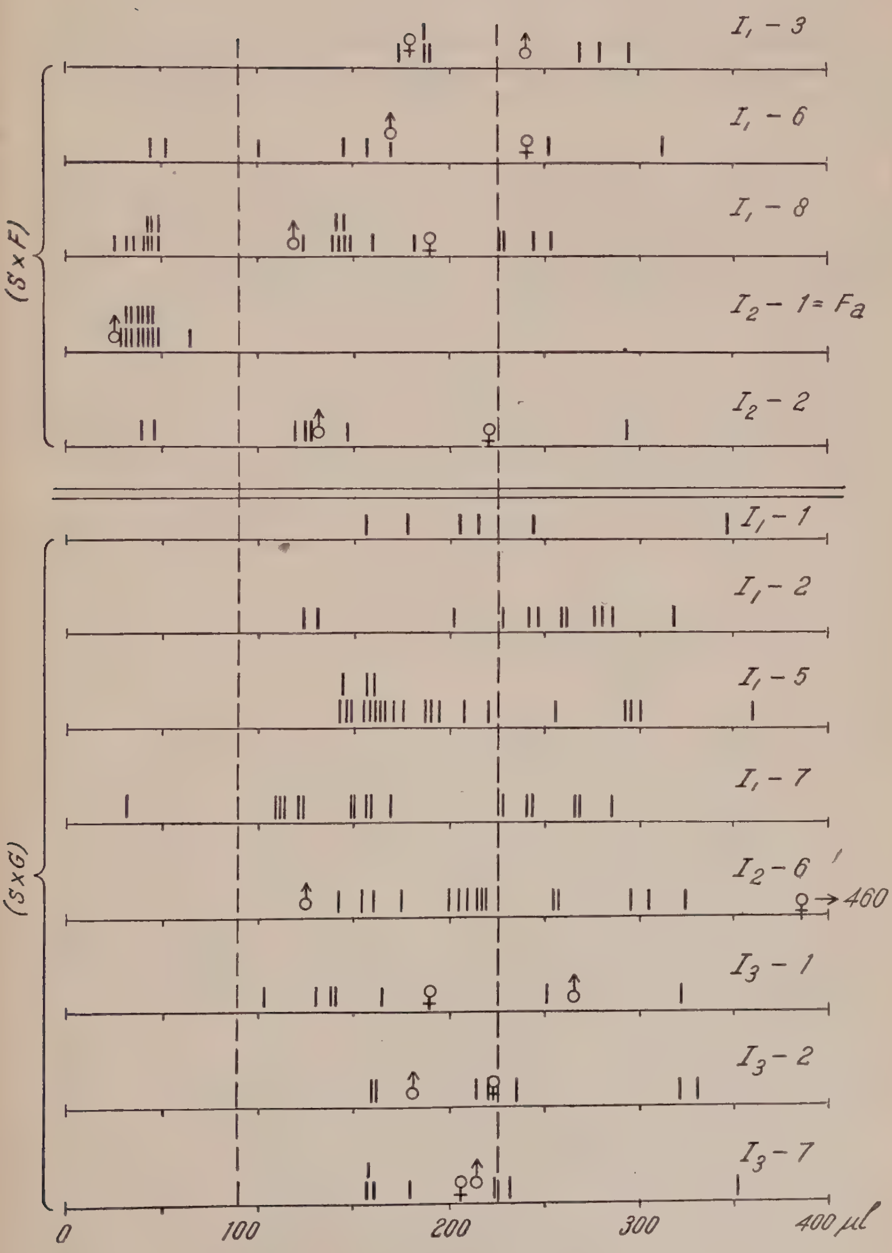

Fig. 6. Inbreeding crosses.

tentatively representing the separation between low and intermediate activity at 90 μl and between intermediate and high activity at 225 μl. It should be noted that some overlap may occur between the intermediate and high groups, so that the place of the second line should not be taken too rigidly.

The segregations found indicate the presence of a single gene for low ali-esterase activity in strain F which will be called *a*, whilst its allele with high activity, representing the wild type will be called a^+. Accordingly the three groups can be written *aa*, aa^+ and a^+a^+. A rather large spread of the values is present in the last two groups, probably due to phenotypic variation and inaccuracy of the method.

The presence of a single gene is further substantiated in the backcrosses B_2—B_4 (Fig. 2—4). Here individual flies with unknown ali-esterase activity belonging to a progeny in which both intermediate and high activities were found, were crossed with S. In this way two kinds of offspring must be obtained, depending on whether aa^+ or a^+a^+ had been crossed with S.

For the study of the ali-esterase activity in the backcrosses generally 7 individual flies were used of the offspring from each single pair. If all had a high activity, the whole progeny was considered to be of the a^+a^+ type; if some had intermediate activity it was apparently a mixture of aa^+ and a^+a^+. It is, of course, possible that an a^+a^+ progeny was wrongly classified, due to the chance that only members of one class had been taken, whereas both were present.

In B_2 6 progenies are present with intermediate and high activity, and 5 with only high activity (The situation in B_2 4 and B_2 7 is uncertain, but they probably represent the high activity type). In B_3 the segregation is 1 of the mixed type to 7 with only high activity, in B_4 4 mixed type and 4 high type (if B_4 8 which is somewhat uncertain is assumed to be of the high type).

From the fact that in the male flies the a^+a^+, aa^+ and *aa* groups are found it can be seen that the *a* gene is autosomal. If it had been on the sex chromosome, only two groups would have been possible.

After the 4 backcrosses the flies were inbred in the crosses I_1 and I_2 (Fig. 6), in which it was tried to reselect a strain with low ali-esterase activity (*aa*). As was also done in some of the backcrosses the activity of the parent flies that were still alive was estimated after they had laid eggs. The estimations of these flies have been designated in the figure as ♂ and ♀ respectively on the same line as their offspring. These flies, often older than 10 days, cannot always be classified according to the 90 and 225 μl lines which have been estimated on young flies only. Especially for the females they may have to be considerably shifted to the right. Nevertheless the activities found made it possible to test only the progenies which were expected to give the best results, viz. I_1 3, I_1 6, I_1 8, I_2 1 and I_2 2 (Fig. 6). The other crosses of this type are not shown in the figure. In this way a homozygous *aa* strain was obtained in two generations. It will be called F_a.

Fig. 5 and 6 show the estimations of strain G and its crosses. A similar situation is found as in strain F. There is the complication, however, that strain G when the crosses were started was not pure *aa*, but contained some aa^+ and a^+a^+ flies, perhaps caused by contamination with flies from another strain (Fig. 5, data of 14/4 '59). Consequently its f_1 and B_1 crosses were not pure. In later estimations strain G was found to consist only of flies with low esterase activity (Fig. 5, data of 3/6 '59), probably as a result of the selection during breeding of the strain. In the backcrosses segregations were as expected. In the crosses I_1—I_3, where brother-sister matings were made for the isolation of the *aa* type, this type was either absent or present in too small numbers. This could perhaps be explained by assuming a lethal factor which showed its effect when the inbreeding was carried out. Consequently it was impossible to obtain the *aa* type after the backcrosses with S. Difficulties of this kind may be avoided by the use of inbred strains, in which the lethal factors can no longer be present.

Resistance

In crosses of S × F and S × G, f_1's were obtained which were slightly less resistant than the resistant parent strains. The difference between the f_1's and S is rather large, and a range of doses can be applied giving 100% mortality in S, and practically zero in f_1 (Table II). If one gene for resistance was present, the backcross with S should give a progeny 50% of which has the resistance level of f_1, and 50% that of S. Based on the figures given in Table II, two discriminating doses were chosen, viz. 4 and 8 μg of diazinon per jar for males and 8 and 16 μg for females in the cross S × F; 2.5 and 5 μg of malathion for males in the cross S × G. In order to obtain as much information as possible from one batch of flies, mortality was counted at 2, 6 and 24 h after the flies were introduced into the contact jars or after topical application.

TABLE II

Difference in percentage mortality between S and f_1 flies.

	diazinon				malathion		
dose μg/jar	♂♂		♀♀		dose μg/fly	♂♂	
	S	f_1 (S x F)	S	f_1 (S x F)		S	f_1 (S x G(
2	98	0			2	98	13
4	100	0	95	3	3.5	100	10
8	100	8	100	5	5	100	13
16	100	25	100	5	7	100	25

Fig. 1 shows the mortality found with these doses for diazinon in S and the f_1 of S × F, and also the mortality which should be found in the first backcross if one gene was present. The corresponding data with malathion in strain G are shown in fig. 5. The results obtained in the first backcross are also presented in fig. 1 and 5. In the cross with F the results are not in accordance with the one-gene concept, in the cross with G good agreement with expectation exists (except in B_1 5 which is probably due to the impurity of strain G, as mentioned before).

The presence of one gene for resistance in strain G is confirmed in the second backcross. Since parents are chosen from the progeny of B_1, consisting of 50% susceptible and 50% resistant flies, the progeny in the B_2 of some pairs is susceptible, (B_2 1, 3 and 5) and of others again devides into 50% resistant and 50% susceptible (B_2 2 and 4).

The presence of more than one gene for resistance in strain F is indicated by the fact that in B_1 generally an increasing mortality is obtained with higher dosages. Moreover the progenies obtained in the backcrosses that are resistant outnumber those that are susceptible. In B_2 only 3 progenies are obtained which are susceptible (B_2 1, 4 and 5) whereas the 7 others all consist partly of resistant flies. In further backcrosses the number of the resistance factors present will depend on the progeny from which the parents for the next generation are recruited. Since in B_3 only 2 susceptible progenies are obtained (B_3 7 and 10) against 6 showing more or less resistance, it is not unlikely that the B_2 8 progeny from which the parents for B_3 were obtained contained more than one factor. Further evidence on the presence of more genes for resistance in strain F will be given below.

Relation between the a *gene and resistance*

In strain G one factor for resistance and one for low ali-esterase activity is present. Since the *a* gene is selected out, it must be a resistance factor. Therefore the two genes must be identical. If the genes are identical, the progenies in the backcrosses in which resistance is found should be the same as those in which intermediate ali-esterase activity occurs. This is the case in B_1, 1, 2, 3, and 4, and in B_2 2 and 4. In the other progenies B_1 5 and B_2 1 and 5 both resistance and the aa^+ type are absent. B_2 3 is susceptible and has some intermediate ali-esterase activities, but since the parent fly had a high activity, these probably are genotypically of the a^+a^+ type. Therefore the low ali-esterase gene and the factor for malathion resistance are identical.

In strain F the presence of more resistance factors is likely. If *a* is a resistance gene, the progenies of the backcrosses containing intermediate ali-esterase activity should show resistance. This was found to be the case (B_2 2, 3, 6, 8, 12, B_3 6, B_4 6, 9, 11, 12). However, consistent with the idea that more genes are involved, resistance was also found in progenies not having the *a* gene (B_2 7, 11, B_3 2, 4, 5, 8, 9, B_4 10). It is curious that the resistance genes other than *a* are still present in B_4. This can be due to chance or to linkage. In B_4 4 progenies are found in which the *a* gene is present. In 3 of these the percentage of resistant flies is very high, indicating the presence of other resistance genes (B_4 9, 11, 12). In one the percentage is low (B_4 6). Since the chance that in this progeny other factors than the *a* gene are present is smallest, it was chosen for the further crosses. By selection for low esterase activity the F_a strain was obtained. Its resistance is shown in Table III. It is probable that this level of resistance is brought about entirely by the a gene.

Starting from a progeny with resistance but without the *a* gene, an attempt was made to obtain a strain with only this other factor (or factors). B_2 11 was such a progeny, and five flies from it were backcrossed with S. In the progeny B_3 14, resistance without the *a* gene was found. However, the unexpected result was obtained, that in 2 others, B_3 12 and 15, intermediate ali-esterase was present whilst the parents had high activity. Unfortunately it was impossible to study whether this was true genotypic aa^+ since the cultures were lost.

Starting from B_3 2, the experiment was repeated. B_4 1—5 were obtained of which 1, 4 and 5 showed resistance and high ali-esterase. B_4 5 was selected with diazinon for 3 generations and a strain Fb with high ali-esterase activity and resistance was the result (Table III). It should be noted that it is possible that strain F contains more resistance factors than those isolated in strain F_a and F_b. The differences found in resistance of some of the B_1 progenies (e.g. B_1 1 and 5) may indicate the influence of more factors present in the heterozygous state.

TABLE III

Susceptibility for diazinon of strains S, F, F_a and Fb

amount of diazinon (μg per jar)	Percentage knockdown after 24 hours' exposure			
	S	F	Fa	Fb
1	26			
2	88			
4	97			
8	100		23	20
16			76	68
32		5	100	100
64		10		
128		63		
256		98		

DISCUSSION

In a paper to be published shortly, Nguy & Busvine (1960) studied the genetics of a malathion and two parathion resistant strains (Am, C and D). One gene for resistance was found in each strain. It was shown that the gene for malathion resistance was located on the same chromosome as the gene for parathion resistance of one of the strains, and probably was an allele of it. Low ali-esterase activity is present in these strains, so that genes causing this low activity and at the same time resistance must be present in at least five strains. These genes cannot be identical because the specificity and/or the degree of resistance in these strains is not the same. This may not apply for strain D and F since their difference may be caused by the action of the other factors present in strain F. Therefore at least four different genes for low ali-esterase activity must exist and there are reasons to believe that they are alleles. First they affect the same enzyme, in such a way that resistance ensues. Second, two of them giving diazinon and malathion resistance respectively (present in strain C and Am) have been shown by Nguy and Busvine to be closely linked or to be alleles. It is, therefore, proposed to indicate them by the symbol "*a*", followed by a suffix derived from the name of the strain: *a*F, *a*G, *a*Am, *a*C, *a*D. As said before, *a*F is possibly identical with *a*D. Further experiments to test the allelism of the genes using ali-esterase activity measurements are under way.

In view of their common influence on the ali-esterase it seems likely that physiologically closely related defence mechanisms are present in the various strains. Unpublished experiments indicate that the resistance factors are responsible for the presence of enzymes, which hydrolyse the oxygen analogues of the toxicants, and which seem to be made at the cost of the ali-esterase (VAN ASPEREN & OPPENOORTH, in preparation). In a previous paper (OPPENOORTH 1959) it was reported that two strains, F and C are approximately equally resistant to a number of organophosphates. Since according to NGUY & BUSVINE (1960) resistance in strain C depends on one gene, this must confer a resistance equal to that brought about by at least two factors in strain F. This is confirmed by the fact that hydrolysis of diazoxon takes place at a much higher rate in strain C than in strain F.

Nothing is known about the nature of the resistance factor isolated in strain F_b. Since this factor is independent of the low ali-esterase gene, it must be responsible for a different resistance mechanism, which in strain F adds to the resistance brought about by the *a* gene. Low ali-esterase activity has been found in all phosphate resistant strains studied, and in four no other genes seem to be of importance. Therefore it appears likely, that genes for low ali-esterase activity are more frequent in *Musca* populations than genes of the kind found in strain F_b.

The method of repeated backcrossing with a susceptible strain, as recommended by CROW (1957) for the analysis of the genetics of resistance appears to be an important tool in the study of the significance of a physiological property correlated with resistance. It is possible to investigate whether one factor is involved, provided that this property can be measured accurately and preferably in single flies. If this is the case the factor can be isolated to determine which fraction of the total resistance depends on it. The importance of resistance mechanisms found could be estimated in this way and the existence of other mechanisms for resistance may be detected.

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ZUSAMMENFASSUNG

GENETIK DER RESISTENZ GEGEN ORGANISCHE PHOSPHORSÄURE-VERBINDUNGEN UND DER GERINGEN ALIESTERASE-AKTIVITÄT BEI DER STUBENFLIEGE

Der Zusammenhang zwischen niedriger Aliesterase-Aktivität und Phosphorester-Resistenz wurde in einem gegen Malathion und einem gegen Diazinon resistenten Stamm von *Musca domestica* mit Hilfe eines Systems wiederholter Rückkreuzungen mit einem normalsensiblen Stamm studiert. In dem Malathion-resistenten Stamm sind niedrige Aliesterase-Aktivität und Resistenz von ein und demselben autosomalen Gen abhängig. In dem Diazinon-resistenten Stamm F ist ein Gen für niedrige Esterase-Aktivität und einen Teil der Resistenz verantwortlich, während mindestens ein weiterer Faktor für Resistenz ohne Einfluß auf die Esterase-Aktivität vorhanden ist. Zwei Stämme wurden gezüchtet: einer, F_a genannt, nur mit dem Gen für niedrige Esterase-Aktivität, und der andere, F_b ohne dieses Gen, aber mit einem anderen Resistenzfaktor.

Da NGUY und BUSVINE in drei Stämmen je ein einziges für die Resistenz verantwortliches Gen gefunden haben, sind nun insgesamt fünf Stämme mit einem wichtigen Gen für niedrige Esterase-Aktivität und Phosphorester-Resistenz beschrieben worden. Diese Gene, die sich nach dem Grade und der Spezifität der Resistenz, die sie bewirken, unterscheiden,

sind wahrscheinlich alle Allele. Es wird daraus geschlossen, daß für diese verschiedenen Arten von Resistenz physiologisch nahe verwandte Abwehr-Mechanismen verantwortlich sein müssen.

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SHORT COMMUNICATION

HERBICIDE SPRAY AND FRIT FLY ATTACK ON OATS

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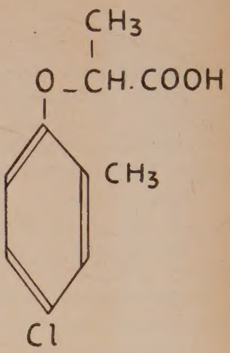
In large scale field experiments on pest insects, it is usually desirable that the crop used should receive all the treatments, other than those which form part of the experiment, customary to good farming practice. Hormone weedkillers are now used almost routinely with many crops, particularly with cereals, and entomologists have often wondered whether they affect insects. The evidence suggests that they are not insecticidal, but in experiments in which the behaviour of insects is being studied, it is desirable to know whether weedkillers affect behaviour by making sprayed areas of crop less or more attractive than unsprayed areas.

In an experiment in which frit fly (*Oscinella frit* L.) behaviour was being studied in oats, a 4 acres block was planted half with the variety Sun II and half with the variety Eagle. Detailed results of this experiment, done in collaboration with members of the National Agricultural Advisory Service in England, will be published later. We are here concerned with the effects of a herbicidal spray on the frit fly.

The oats were sown on 11 April 1959 (this is later than is normally recommended as we wished to encourage a high frit-fly infestation). On 12 May about a third of the area planted with each variety was sprayed with the herbicide CMPP at the rate of 4 pints in 40 gallons of water per acre. CMPP is α-2-methyl 4-chloro phenoxypropionic acid and is recommended particularly for killing annual weeds, though it is also fairly effective against some perennial weeds. Later examination of the field showed that weeds, particularly perennial creeping thistles, were much less common on the sprayed than on the unsprayed areas.

Frit fly appeared in the field early in May and had begun egglaying on the oats by 12 May, when the herbicide was sprayed. Dissections of oats from the sprayed and unsprayed areas were made on 17 June, when tiller damage from frit fly had reached a high level. Five samples, each of 30 plants were taken from different parts of the unsprayed and sprayed areas of the two varieties. Results of dissection were as follows:

	Infested tillers per 30 plants	
	Unsprayed	Sprayed with Herbicide
Variety		
Sun II	25 ± 9	35 ± 17
Eagle	18 ± 7	34 ± 16



CMPP

These results show that the herbicide neither killed nor repelled the frit fly. Indeed the infestations of the sprayed area were higher than the unsprayed, but the difference is not statistically significant. Other examinations made in the field support the view that the weedkiller had no effect on the behaviour or survical of the frit fly, but it is desirable that many more experiments in the effects of herbicides on insects should be made.

ZUSAMMENFASSUNG

HERBIZID-SPRITZUNG UND FRITFLIEGENBEFALL AN HAFER

Am 11. April gesäter Hafer (Sun II und Eagle) wurde am 12. Mai mit dem Herbizid CMPP gespritzt, zu einer Zeit, als Fritfliegen im Bestand vorhanden waren. Das Herbizid hatte keine Wirkung auf das Verhalten oder die Sterblichkeit der Fritfliegen.

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